The Synthesis of bis-(2-Chloroethyl)-Methane Phosphonates

Thesis

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The Synthesis of bis-(2-Chloroethyl)-Methane Phosphonates

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Abstract

This thesis describes attempts to synthesise phosphoryl carbon mustards (bischloroethylphosphonates). Several strategies were employed based on phosphorus-carbon or carbon-carbon bond disconnections. Thus routes involving the reaction between:

(i) phosphites, nucleophilic phosphorus reagents, and haloalkanes or ketones;

(ii) dialkyl chlorophosphates, as electrophilic phosphorus reagents, and carbon nucleophiles; and

(iii) nucleophilic dialkyl alkanephosphonate anions and carbon electrophiles are described.

Thus far none of these routes yielded the desired compounds and some reasons for this are discussed.

Acknowledgements

I would like to thank Dr J.Iley for his supervision and guidance throughout this work.

In addition, I would like to thank the academic and technical staff, and in particular the technicians, Jim Gibbs, Gorden Howell and Pravin Patel without whose help, this project would have been more difficult.

To the Unknown Intermediate.

Declaration

All the work presented in this thesis was carried out by myself in the Department of Chemistry, Open University, Milton Keynes. This work has not been accepted for any other degree, nor is it being currently considered in candiature for any other degree.

David John Woodnutt

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Abbreviations

The following non-standard abbreviations have been used throughout this dissertation.

Abbreviation		Expanded Form
C	-	Carbon.
DNA	-	Deoxyribonucleic acid.
HN2	-	Nitrogen Mustard.
Hz		Hertz
IR	-	Infrared
NMR	-	Nuclear Magnetic Resonance.
P	-	Phosphorus.
Sn1	-	Substitution Nucleophilic Unimolecular.
Sn2	-	Substitution Nucleophilic Bimolecular.

Spectroscopic data:

b	-	broad peak
d	-	coupled proton resonance peak giving a doublet
dd	-	a doublet of doublets
dt	-	a doublet of triplets
dq	-	a doublet of quartets
J	-	Coupling constant
m	-	coupled proton resonance peak giving a multiplet
m/e	-	mass to charge ratio
NOE	-	Nuclear Overhauser effect
ppm	-	parts per million
q	-	coupled proton resonance peak giving a quartet
s	-	single proton resonance peak
t	-	coupled proton resonance peak giving a triplet

Part I: Introduction

Introduction

I.1 The Need For Anti-Cancer Drugs

I.1.1 The Disease

Cancer is not a disease in itself but more a class of widely varying diseases which have in common the uncontrolled and rapid proliferation and growth of cells. No organism or organ is immune from cancer which differs from most other disorders in that it has the ability to invade and destroy normal tissue with there being little defensive response from the host.

For reasons which are poorly understood, a cell, following certain 'initiation' and 'promotion' stimuli, may embark on a course of metaplastic, dysplastic and, eventually, frankly malignant change. This will result in the rogue cell losing contact inhibition of growth offered by the surrounding tissues and a concomitant loss of function - a process which can be thought of as 'de-differentiation', as the cell takes on a more evolutionary primitive morphology.

In time, a tumour (also known as a neoplasm) will form, consisting of a mass of malignant cells and its stroma (the supporting connective tissue framework). Alternatively, if the initial cell is mobile, such as a blood cell, an increase in rogue cell concentration will occur systemically instead of a fixed tumour (*e.g.* the leukaemias).

Malignant diseases not only grow by expansion of the tumour but can also infiltrate both neighbouring and distant tissues. Tumour cells can become detached from the main mass and, often with the aid of the host's own transport systems, may become lodged at distant sites. At these sites, the

malignant cells may settle and grow to give further tumours which are termed 'secondary tumours' or 'metastases.' The degree to which this occurs and the sites at which the cells metastasize to, depends on, and is sometimes characteristic of, the primary tumour. When the cancer has metastasised, the organism is said to have disseminated cancer and this usually represents an advanced stage of the disease.

I.1.2 Control of Cancer

Such malignant diseases, as with all diseases, require control. Moreover, it is desirable to find and remove the cause as well as to find a cure. Considerable success has been achieved in cancer treatment in recent years. This is largely due to the introduction of chemotherapy in conjunction with the more traditional forms of treatment.

The success of this combination therapy is illustrated by the prognosis offered by the treatment of a rare cancer, choriocarcinoma, which is derived from placental tissue. With surgery alone, the disseminated disease has a negligible cure rate but following the use of the antimetabolite, methotrexate and the antibiotic, actinomycin-D, 80% - 90% cure rates have been achieved.[1] When it is considered that most sufferers are young mothers, this is a particularly good achievement.

Because of this success, which is shared by that now attained with Burkett's lymphoma, it has been postulated that the actions of the cytotoxic reagents is to lower the tumour bulk to below some critical threshold when the host's unusually high immune response which is evoked by these tumours, results in the tumours complete eradication[2]. Unfortunately, few types of tumour do

evoke such a significant immune response and this may account for the higher relapse rate seen with most other chemotherapeutically treated malignancies.

Typically, today, surgery is performed on the primary neoplasm followed by high energy radiation (*e.g.* γ–rays) of the surrounding area to eradicate any remaining malignant cells. Concurrently, chemotherapy is administered to eliminate distant metastatic accumulations which may be present. (Metastasectomy alone is rarely effective because of the difficulty in locating the loci).

I.1.3 Anti-Cancer Chemotherapy

The first reported use of a chemical to treat a malignant disease was of a surgeon, Bilroth, who administered arsenic to a patient who had Hodgkin's Disease over a hundred years ago but little became of it because of arsenic's lethal toxicity.

Following the observation that mustard gas (yperite) induced leukopaenia (too few white blood cells) during the Great War, thought was directed towards its use to treat leukaemia where there is an undesired leukocytosis (too many white blood cells). Unfortunately, this was largely ignored until a revival of interest in toxic gases occurred during the Second World War.

I.1.3.1 Problems with Toxicity

In the following ten years, over three thousand alkylating agents were synthesised for evaluation as potential chemotherapeutically active agents but fewer than a dozen survive today. Like radiotherapy, anti-cancer drugs are

cytotoxic to normal cells as well as to malignant cells and considerable problems arise because of this in the administration of the drugs.

To try and overcome this, an increase in specificity can be achieved by the design of the drug. For example, some agents are administered to the body as a pro-drug which is an inactive precursor capable of being broken down to the active form of the drug in the locality of the tumour.

Another common method used to increase malignant cell specificity is for the drug to have a relatively short half-life within the body, it being administered upstream intraarterially (or intravenously) in close proximity to the tumour. This maximises the dose of the drug to the area of the malignancy but at the same time reducing the quantity of the drug reaching the normal tissues. Nitrogen mustard is still used in this way today for emergency relief of superior vena cava obstruction caused by such tumours as bronchial carcinoma.

There is also considerable expectation that a practical form of the immunoglobulin directed drug treatment can be developed which will enable anti-cancer drugs to reach their target with almost 100% specificity: the so called 'magic bullet'.

I.1.3.2 Dose Regimes

The effectiveness and acceptability of chemotherapy was increased by two important discoveries in the method of drug administration.

First, it was found that high-dose periods interspaced with passive periods (in which no drug is administered) proved to be a far more effective use of anti-

cancer drugs against malignant cells, with less toxicity to normal tissue. This selectivity arises from the property that malignant cells rapidly divide and so are more susceptible to mitogens; less rapidly dividing cells such as in most normal tissue, will have a greater proportion of cells in the resting phase of mitosis and so are correspondingly less affected by the phase specific mitotoxins. This form of treatment is known as 'pulse treatment'.

Secondly, it has been found that a combination of anti-cancer agents has a synergistic efficacy over the expected effect of the sum of the single treatments. This selectivity allows lower doses of individual drugs to be used and hence the lower incidence of side-effects including the toxicity towards normal tissue. This results in a higher Therapeutic Index (TI)[3] which leads to an increase in the available dose.

'Pulsed, multiple drug' therapy has had an immense effect on the response to treatment of many forms of cancer such as with multiple myeloma, for example. Here, a complex regime of varying doses and time scales is employed using the drugs cyclophosphamide, adriamycin, bischloroethylnitrosourea (BCNU) and melphalan. This treatment gives up to 50% of patients a 100% increase in median survival.[4] The consequence of combining this chemotherapeutic regime with radiotherapy has also had a marked effect on the survival in patients with some solid tumours such as small (oat) cell carcinoma of the lung[5]. See Table 1.

Although there is an marked improvement in median survival, there is no evidence to suggest an increase in survival above 15 months even for the limited, staged disease. However, patients complaining of pain, cough and dyspnoea, haemoptysis, dysphagia and superior vena cava obstruction commonly become asymptomatic following chemotherapy which raises the quality of life and makes this important palliative treatment.

Therapy	Complete responses (%)	Median survival (months)	1-year survival (%)
Placebo	0	2.5	5
Radiotherapy	-	6	20
Chemotherapy (single agent)	1	5	18
Chemotherapy (combination of two, three or four drugs)[6]	15	8	25
Chemotherapy (combination of three or four drugs)[6]	23	9	40
Radiotherapy plus combination chemotherapy	31	11	47

Table 1

Complete response and survival in small cell carcinoma

However, an intriguing complication of chemotherapy with these types of cytotoxic drugs may be emerging. With the increased survival of many cancer sufferers, it has become apparent that they have a greatly increased risk of developing a second primary tumour which is unrelated to the first. This gives rise to two possibilities: first, the increased risk represents the natural history of the disease such as a defect in the host's immune system which, because of the death of the patient, has not previously been seen before; or secondly, it may be due to the mutagenic properties of the cytotoxic drugs. To date, neither has been proved sufficiently convincingly over the other but as treatments improve, the question is going to gain in importance.

Chemotherapy has been most successfully used against high grade carcinoma[7] type cancers but it must be emphasised that only certain forms of cancer are susceptible to chemotherapy and in those which are sensitive, each form requires its own particular cytotoxic drugs and combination regimes. Additionally, it must be remembered that these drugs are all cytotoxic to

differing degrees and are usually very unpleasant both in the short term and in the long term to the patients. The need for more types of anti-cancer agents is, therefore, obvious and a great deal more research is needed in their development.

I.2 The History of the Development in Anti-Cancer Agents

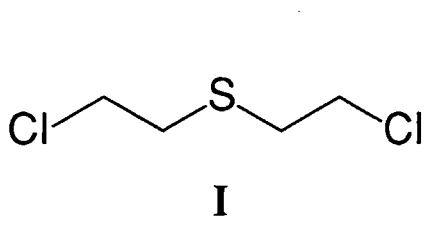
The following history is not an exhaustive account of the development of these drugs but more an introduction and explanation to the modern anti-cancer drugs. It must also be born in mind that many other discoveries were needed in parallel to those in the anti-cancer research; the elucidation of the secondary structure of deoxyribonucleic acid by Watson and Crick in 1953,[8] for example. The apparently slow rate of development of this type of drug is not a reflection of the applied effort.

I.2.1 Pioneer Work

In 1925, Dustin[9] published his findings on cells treated with mustard gas (I) and certain other similar agents: it appeared that the toxicity of these compounds only manifested itself towards the nucleus, the cytoplasm being left unperturbed.

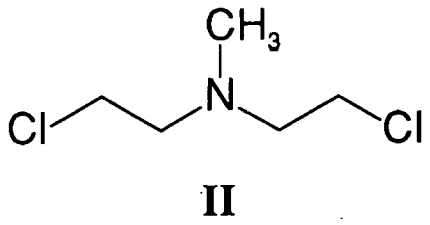
He called these agents, 'poisons caryoclasique', but this was later changed to the more general, 'mitotic poisons', after it was noted that the rapidly proliferating cells were the most vulnerable. His discovery sparked off the start to anti-cancer chemotherapeutic chemistry which eventually led to nitrogen mustard (N,N-bis-(2-chloroethyl)methylamine (II)) being

administered, with a degree of success, as the first anti-neoplastic drug in the 1940's.



I

Mustard Gas



II

Nitrogen Mustard (HN2)

Fig. 1

Following his early discoveries, many studies were undertaken which led to the identification of a whole range of compounds with similar effects but it wasn't until the late 1940's that the chemistry started to be brought together and consolidated. In an article in Nature,[10] the compounds were classified and the mustards and similar compounds were given the title of 'radiomimetric mitotic poisons', so called because of the observed effects of these drugs on the nucleus was considered similar to that seen after X-ray irradiation.

There were differences though: mustard treatment appeared to induce irreversible hereditary changes or mutations which persisted after mitosis in the daughter cells. The deficient or unbalanced genetic material of the daughter cells so affected either caused cell death or at least gave them a reduced viability.

Ford[11] with his work on *Vicia faba* (the broad bean) had demonstrated that the root tip cells (*ie*: those undergoing rapid division) after being treated with nitrogen mustard (II) exhibit chromosome breakage biased towards particular regions of the chromatids - the so called hot spots. He concluded that the

mustards probably bound directly to the acidic sites of the nucleic acid polymer.

I.1.2 Common Factors in Agents with Anti-Cancer Activity

By 1949, vast numbers of widely varying compounds had been shown to have some form of anti-cancer connection. Goldacre *et al*[12] proposed that two certain features had to be present in these compounds for them to exhibit anti-tumour activity: two halogenated groups were required and the halides must be greater than a certain minimum reactivity. Haddow *et al*[13] clearly demonstrated these requirements in an elegant series of comparisons. See Fig. 2.

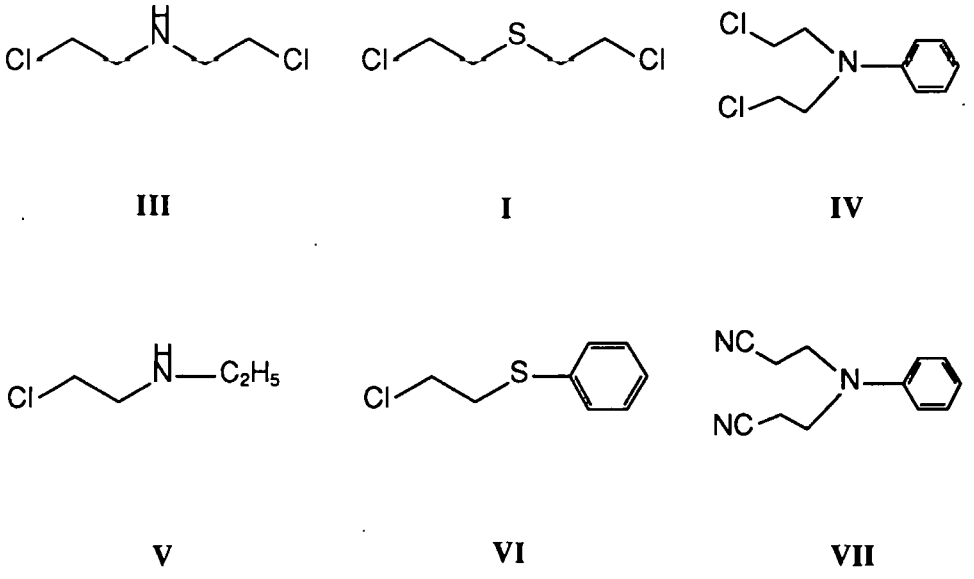


Fig. 2

Compounds I, III & IV (mustard gas, nor-nitrogen mustard and N,N-bis-(2-chloroethyl)aniline), were all previously shown to have anti-tumour activity.

However, compounds V and VI, which are mono-functional analogues of III and I respectively, were found to be inactive. Compound VII (N,N-bis-(2-cyanoethyl)aniline) which has had the two halides replaced by nitrile groups, is also inactive. In addition, substituted analogues of IV which have a deactivated ring (*eg:* *p*-NO_2, *p*-Cl, *p*-CHO, *p*-CO_2Et) all have a reduced activity because of the reduced electron density of the nitrogen's lone pair of electrons. (This lone pair can play a vital role in the activation of the halide as will be described later.) In fact, the work went as far as defining the activity of these compounds as a function of halogen reactivity and compound solubility. This was clearly demonstrated by the xenylamine[14] series where increasing reactivity of the halogen more than compensates for a decreasing solubility of the compounds. See Fig. 3.

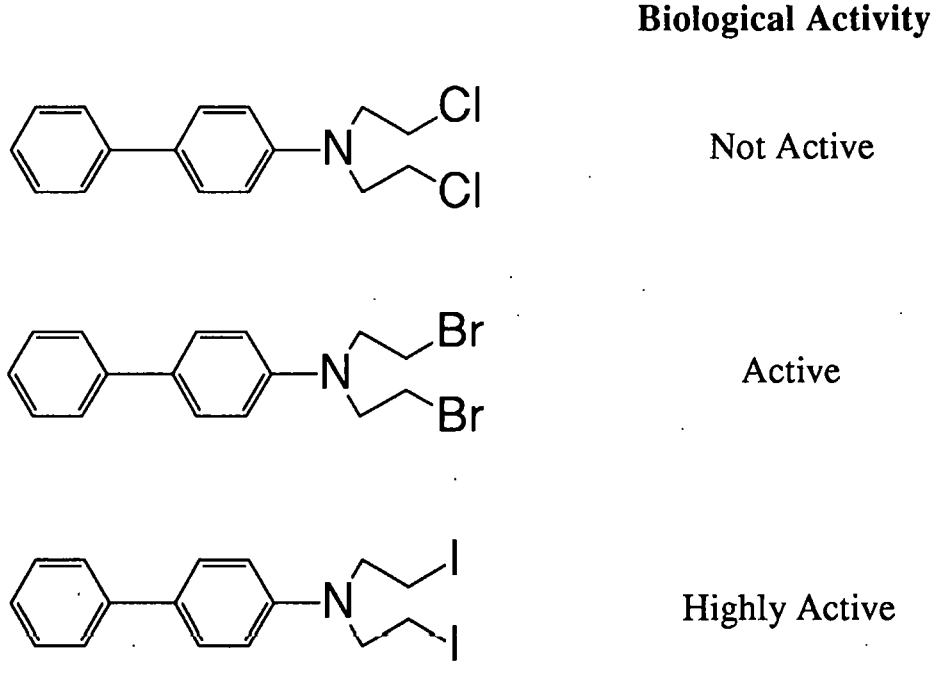
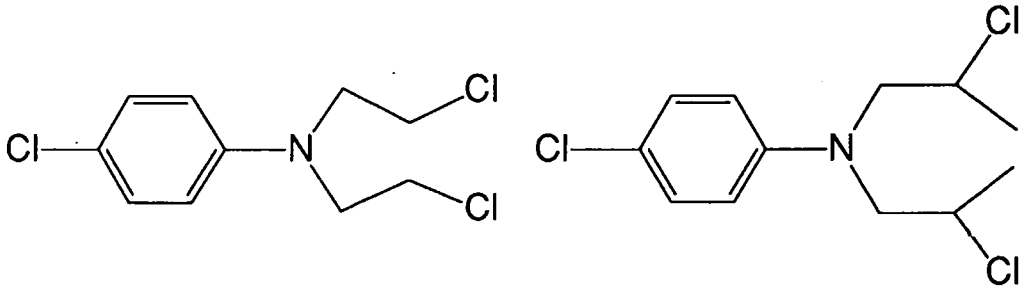


Fig. 3

It was also noted that an increase in toxicity accompanied the increase in anti-tumour activity. While 3-chloropropyl mustards were found to be inactive because of the low reactivity of the halogen, 2-chloropropyl mustards were, in

general, found to be active and with increased toxicity. This is demonstrated by comparing VIII, N,N-bis(2-chloroethyl)-4-chloro-aniline (inactive) and IX, N,N-bis(2-chloropropyl)-4-chloroaniline (active). Presumably, this is related to the ease of carbonium ion formation. The structures of VIII and IX are seen in Fig. 4.



VIII IX

Fig. 4

An insight into these agents' biological mode of action was obtained from Elmore's[15] observation that cells exposed to mustard gas exhibited cross-linking of nucleic acids by their phosphoryl groups (see Philips).[16] This provided an explanation for the necessity of a bi-functional alkylating moiety concluded by Haddow,[13] and the cytological effects described by Kollar,[17] Robson[18] and Allsopp.[19] These chromosome bridges can be clearly seen during the anaphase of mitosis when the paired chromosomes split and migrate to opposite poles of the cell; the cross links formed by the mitotic poisons are also known as 'sticky chromosomes'. In themselves, sticky chromosomes do not directly infer that mustards cross-link DNA., For instance, monoalkylating agents, such as X (Fig.5), where the aziridine group is the only active moiety, also cause chromosome bridges. Philips[20] pointed out, however, that concentrations of up to 50 times that of the bi-functional alkylating agents were needed to give equivalent results.

More direct evidence of their cross-linking comes from analogous studies with mustard gas (I). DNA treated with mustard gas becomes more viscous and a greater number of titratable groups are blocked than mustard residues bound.[15,21] With nitrogen mustard,[22] however, a decrease in thymonucleate viscosity is found.

2-Ethylenimino-4,6-dimethoxytriazine

X

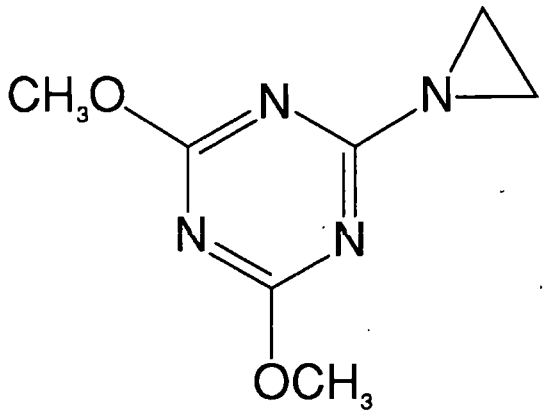


Fig. 5

By 1965,[23,24] the theory of cross-linking was accepted but it is now known that alkylation occurs not at the phosphoryl groups of the nucleotides but at other nucleophilic centres such as the N-7 position on the purine base, guanine. With suitably close guanine residues on the two complementary chains, cross-linking may occur but alkylation of a single strand following separation is enough to disrupt transcription. (However, cross linking, as opposed to alkylation at a single site, is less susceptible to the cellular repair mechanisms and so are more effective antimitotics). Protein synthesis is also disrupted by direct inhibition of the enzymes involved in translation.[25] However, the exact mechanism of alkylation is still unclear.

I.3 ALKYLATING ABILITY OF MUSTARDS

I.3.1 Simple Mustards

A primary halohydrocarbon is a relatively inert functional group. It will reluctantly undergo displacement reactions which mostly proceed by way of an S_N2 mechanism. See Fig. 6.

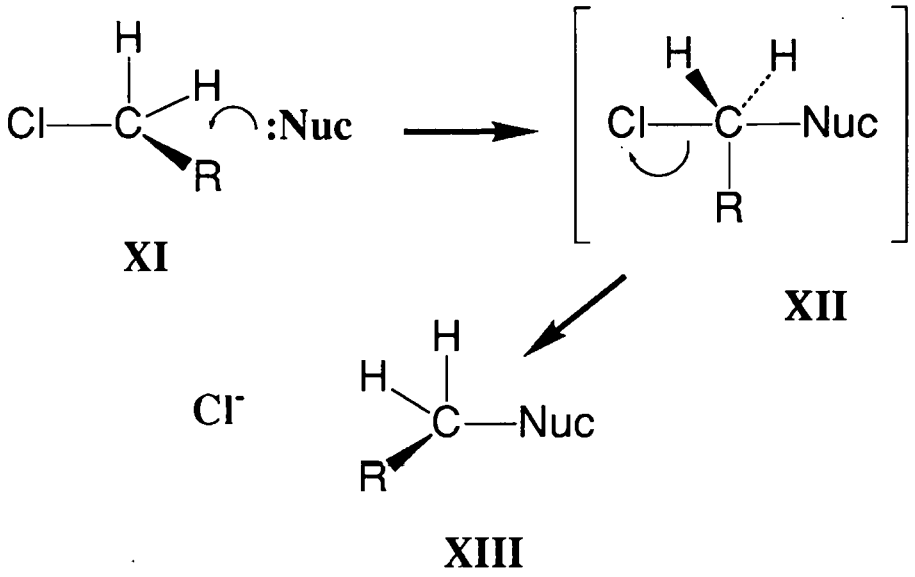
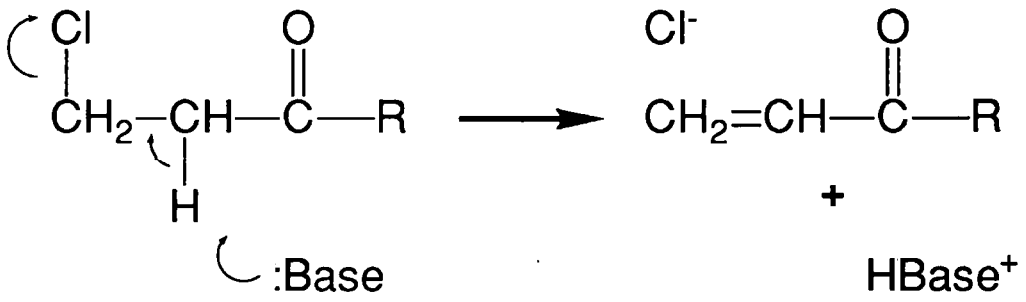


Fig. 6

The more bulky the 'R' group is, the greater the difficulty of the approach for the nucleophile and also the non-bonding interactions of the transition state, XII, which together raise the activation energy, and thus, the reaction proceeds at a slower rate.[26] However, if certain functional groups are spatially arranged in favourable proximity, the halogen can become activated, such as in a β-haloketone which will readily undergo β-elimination by way of an E2 mechanism. The production of an α,β-unsaturated ketone makes the reaction thermodynamically favourable because of the conjugated π-system, while the acidity of the α-hydrogens to the carbonyl group insures that the kinetics will be fast. See Fig. 7.

For mustards, with respect to alkylation, the reaction centre carbon has moderately bulky groups attached: the chlorine and the substituted ethyl moiety. These groups have the effect of reducing the viability of an S_N2 mechanism. However, substitution of the halides can also proceed by way of an S_N1 reaction mechanism.



XIV **XV**

Fig. 7

Base induced β-elimination of 2-chloroethyl moiety.

An S_N1 mechanism is normally disfavoured in a primary haloalkane because of the formation of a relatively unstable intermediary carbonium ion, but under certain conditions the carbonium ion can be stabilised enough to make the S_N1 pathway feasible. Such a stabilising situation may arise from the charge being transferred to an intramolecular heteroatom, such as sulphur or nitrogen which can accommodate a positive charge whilst maintaining a full octet of electrons. This accelerates the expulsion of the halide ion and retains a sufficiently reactive intermediate to encourage attack from the nucleophile.

This effect is called a 'neighbouring group assistance' or an 'anchimeric effect' by the heteroatom. The pathway is shown in Fig. 8.

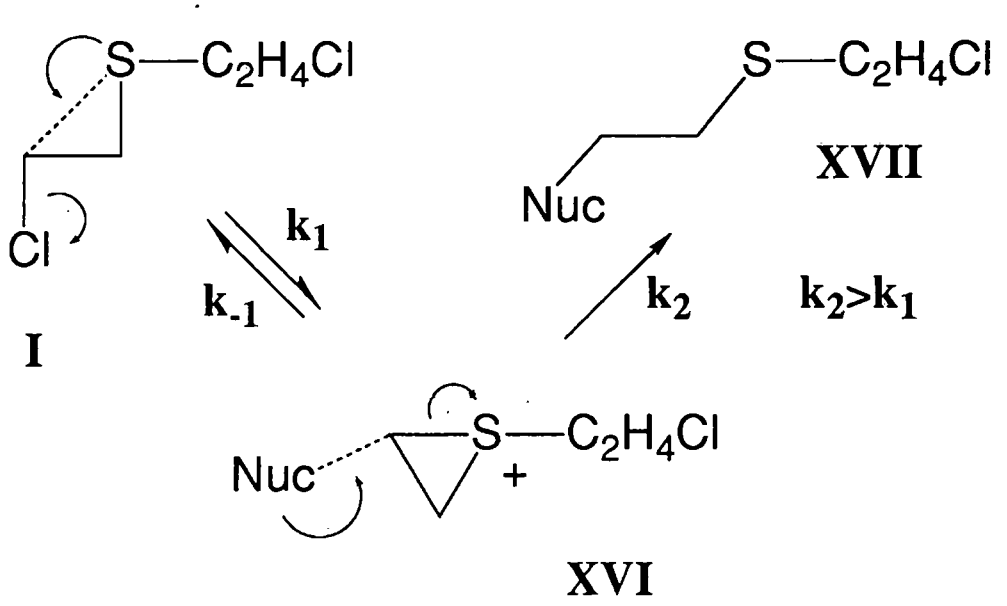
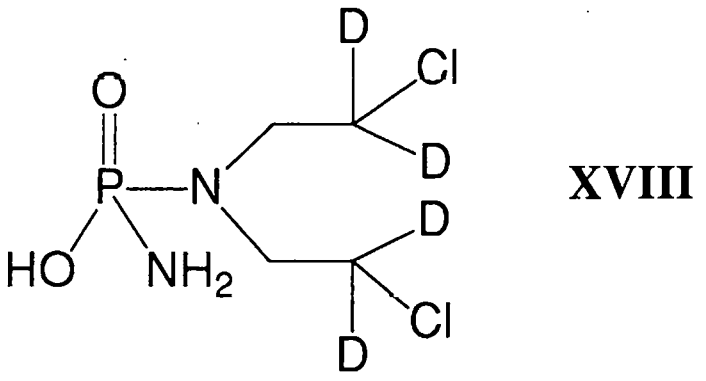


Fig. 8

The scrambling of the deuterium atoms attached to a β-carbon in a labelled mustard, XVIII (Fig. 9), demonstrates this mechanism. See Fig. 10.



XVIII

Fig. 9

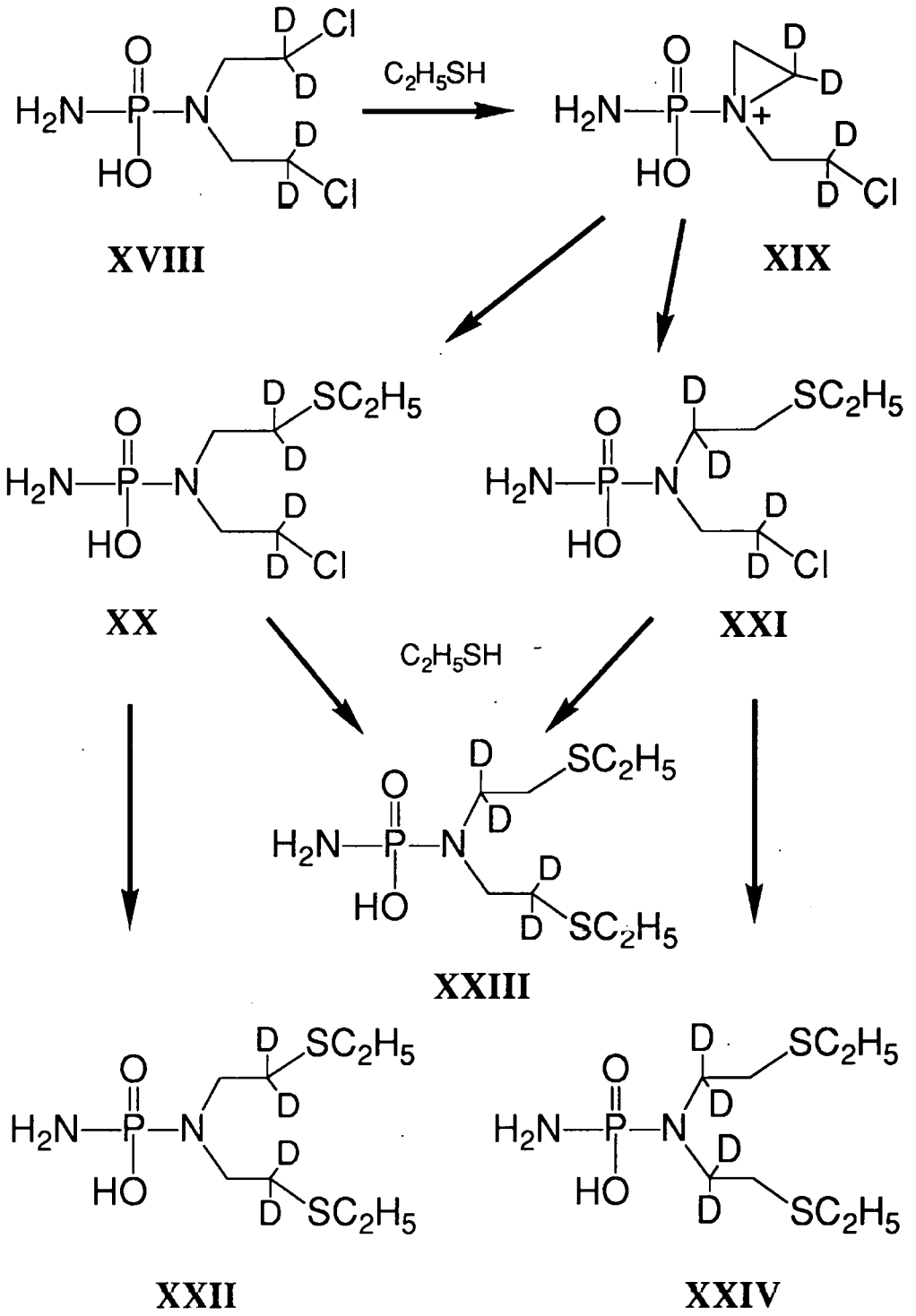


Fig. 10

Colvin[27] found by mass spectroscopy that the bis-alkyated products, XXII-XXIV, were all present in 1:2:1 ratios which could only have arisen via symmetrization of the intermediate aziridinium ions.

The stabilisation of the intermediate by the anchimeric effect offered by either nitrogen or sulphur reduces the overall activation energy by many orders of magnitude giving mustards the sufficient reactivity at 37°C to make them biologically active against tumour cells.[28,29] The degree of rate enhancement that can be obtained is illustrated with three series in Table 2.

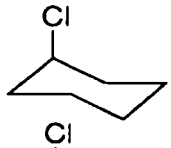
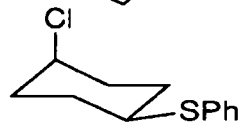
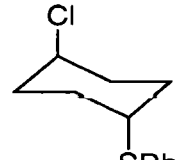
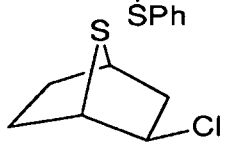
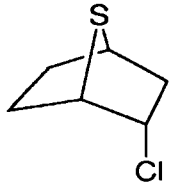
Compound	Relative Rate of Solvolysis		Reference
$CH_3-C_2H_4-Cl$	1.00	H_2O 50°C	30
$CH_3-S-C_2H_4-Cl$	2.93×10^5	H_2O 50°C	31
	1.00	80% Ethanol 118°C	32
	0.16	80% Ethanol 118°C	32
	7.20×10^4	80% Ethanol 118°C	32
	1.00	Acetic Acid 25°C	33
	4.70×10^9	Acetic Acid 25°C	33

Table 2

From Table 2, it can be seen that the molecules which do not allow the correct geometry for the atomic orbital containing the lone pair of electrons from sulphur to have an anchimeric effect, have a correspondingly low rate of solvolysis, but those which allow a favourable interaction have greatly enhanced rates. However, the formation of a stabilised cation is not an

absolute prerequisite and it must be remembered that the requirement is for an activated, or more precisely, an enhanced polarisation of the carbon-chlorine bond. Therefore, the alkylation of nitrogen mustards does not necessarily have to proceed through an aziridinium ion intermediate, and surprisingly, alternative pathways were only considered in earnest as late as 1982 by Zon *et al.*[34] These possibilities are discussed in the next section.

I.3.2 Alternative Mechanisms of alkylation for Nitrogen Mustards

One alternative mechanism for alkylation was proposed by Williamson[35]. Under conditions of mild base, nor-nitrogen mustard reacts with carbon dioxide to give a cyclic carbamate. See Fig. 11. The step of interest is the displacement of a chloride ion by the carboxylate group as opposed to the nitrogen's lone pair of electrons.

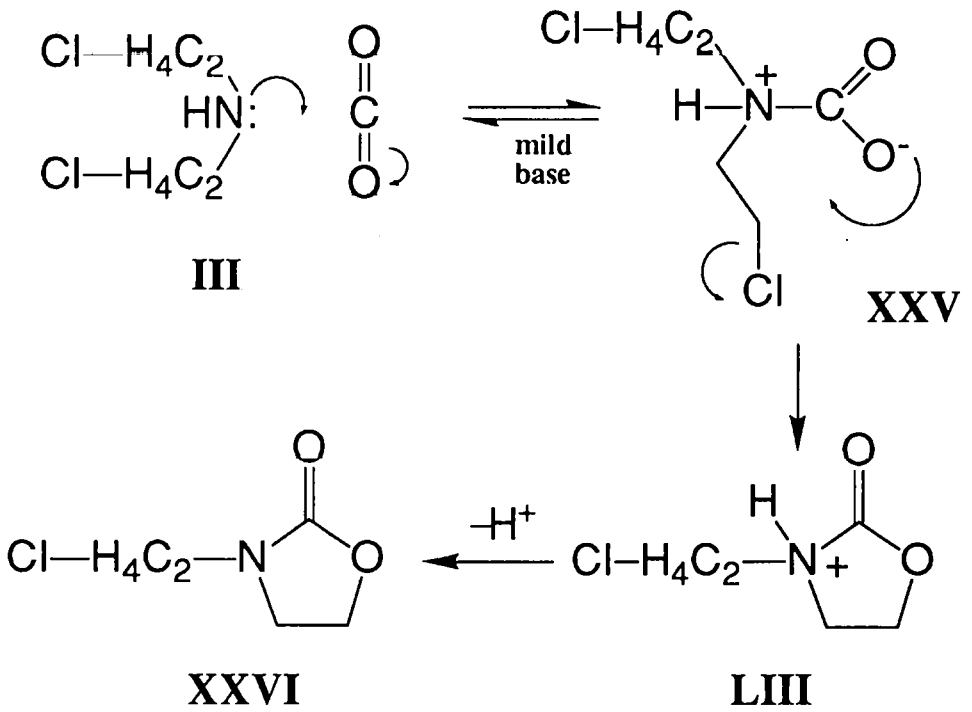


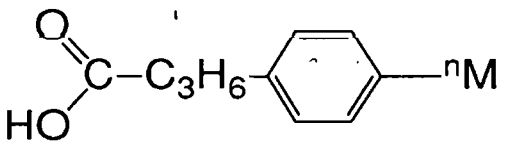
Fig. 11

This idea of mustard alkylating activity without the involvement of an aziridinium ion intermediate will be returned to later in the discussions on cyclophosphamide.

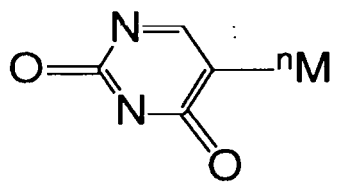
I.4 Cyclophosphamide

I.4.1 Why Cyclophosphamide?

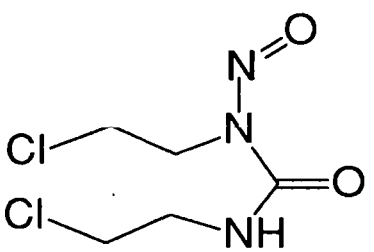
As was discussed earlier, the essential requirements for a successful anti-cancer agent are the 2 chloroethyl groups attached to a heteroatom, such as sulphur or nitrogen, to give the chlorines a sufficiently high reactivity. Compounds III and IV in Fig. 2 together with those shown in Fig. 12 have been found to be biologically active and have all been used, with varying degrees of suitability, as anti-cancer agents: only their poor specificity contributing to their restricted applications.



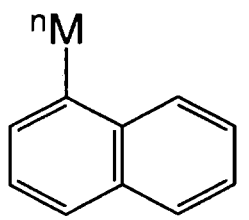
XXVII



XXVIII



XXIX



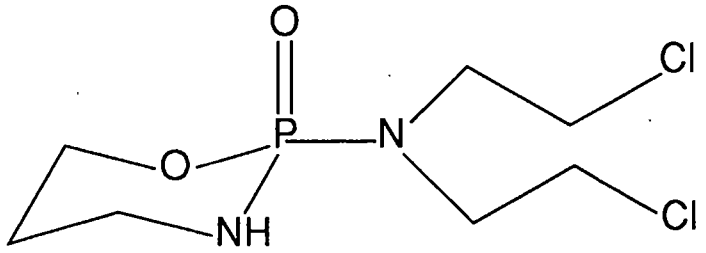
XXX

^{n}M = Nitrogen Mustard Moiety

Fig. 12

Following the discovery by Gomori[36] that neoplastic cells have an increased phosphorylase[37] activity, it was proposed by Friedman[38] to synthesize a phosphorylated nitrogen mustard. The idea being that if the compound was to be administered in this form, the lack of basicity with the attached phosphoryl group would render it devoid of the usual mustard characteristics.[39] Once inside the cell, phosphorylase activity would release the mustard group as nor-nitrogen mustard (III) effectively activating the drug. The specificity shown towards neoplastic cells would, therefore, allow the effective dose to be lowered thus reducing the drugs overall cytotoxicity: in other words, the Therapeutic Index[3] (T.I.) will be raised.[40]

Although the original philosophy was found to be wrong, the phosphorylated nitrogen mustards have been some of the most successful anti-cancer drugs and many hundreds of analogues have been synthesized. By far the most popular skeleton is phosphordiamidic acid and, in particular, cyclophosphamide has been studied intensively since its first synthesis by Friedman in 1954.[38] See Fig. 13.



XXXI

Cyclophosphamide

Fig. 13

Two important factors in the drug's functionality had to be elucidated: (i) its metabolic pathway and identification of its active form; and (ii) the mechanism of alkylation that is followed *in vivo*. These did not turn out to

be easy questions to answer and some possibilities are discussed in the following sections.

I.4.2 Metabolism of cyclophosphamide

Much light was thrown on the metabolic pathway of cyclophosphamide mustard (XXXI) Colvin *et al* in 1973[43] See Fig.14. The detection of acrolein (XXXVII) and phosphoramide mustard (XXXVI) in the plasma and urine of patients treated with cyclophosphamide[42-5] helped solve the apparent anomaly that despite cyclophosphamide's highly cancerotoxic activity *in vivo*, it shows only low biological reactivity *in vitro*. Phosphoramide mustard, however, is known to be a good alkylating agent.[46] Hence, cyclophosphamide appeared to be a prodrug and this helps to explain its high therapeutic index. See Table 3. Thus, with a large therapeutic index, larger doses can be administered and so makes the treatment more effective. (See section I.1.3)

More recently, phosphoramide mustard has been found to be a potent alkylating agent at physiological pH: phosphoramide mustard reacts with sulfhydryl groups,[27] guanosine,[47] guanosine 5-monophosphate,[48] deoxygaunosine[47] and phosphodiester groups in DNA.[49] Phosphoramide mustard has also been found to cause both DNA-protein and intrastrand DNA cross-links.[50] The relatively high oncostatic specificity of cyclophosphamide mustard *in vivo* was shown to be due to the cytotoxic specificity of 4-hydroxycyclophosphamide (XXXII),[51] the first product of the metabolic activation of cyclophosphamide mustard in the liver. In itself, XXXII is not an alkylating agent but attains this property only by release of phosphoramide mustard.

The formation of phosphoramide mustard is driven by the accompanied formation of the resonance stabilised acrolein (XXXVII).

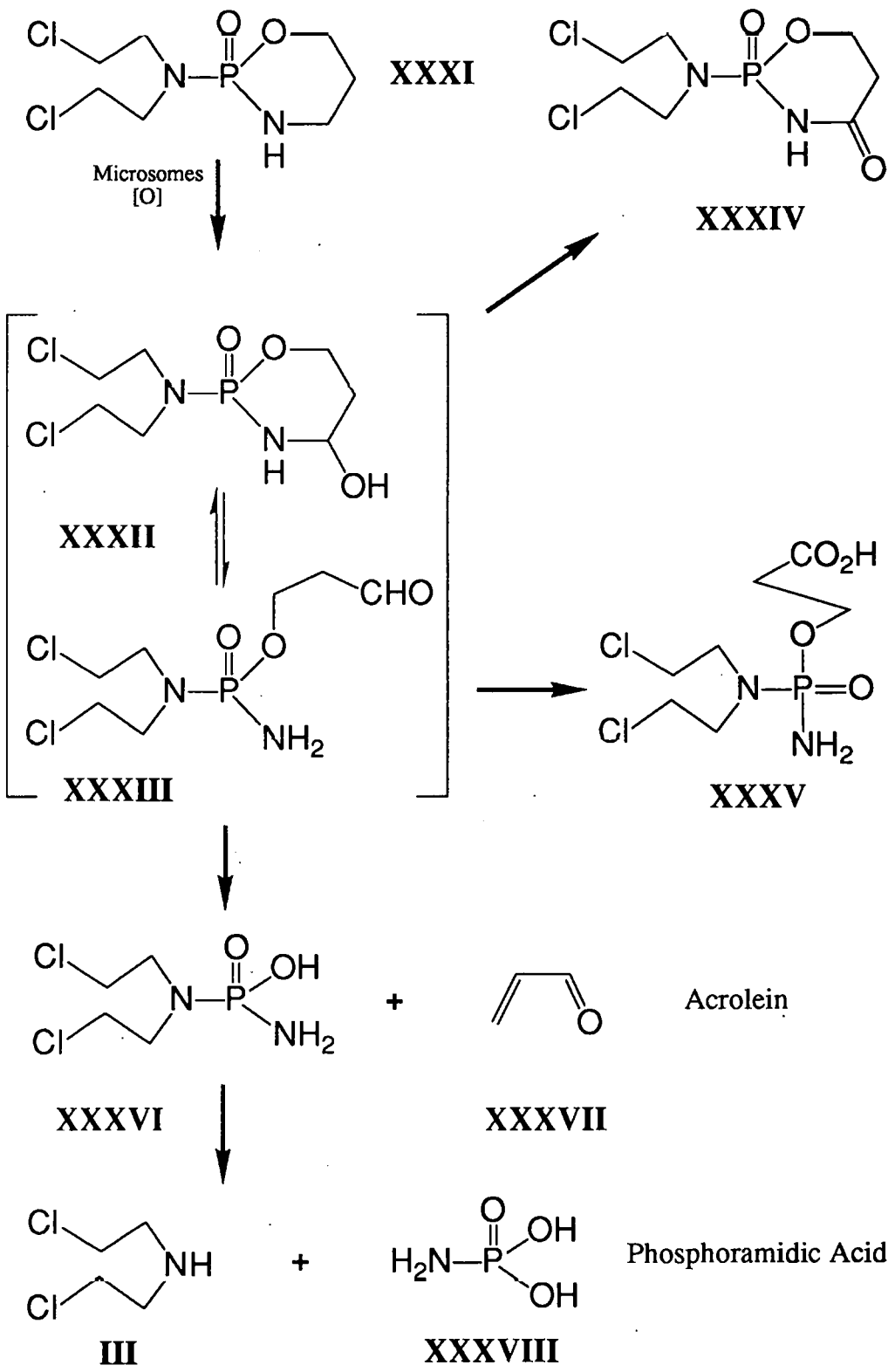


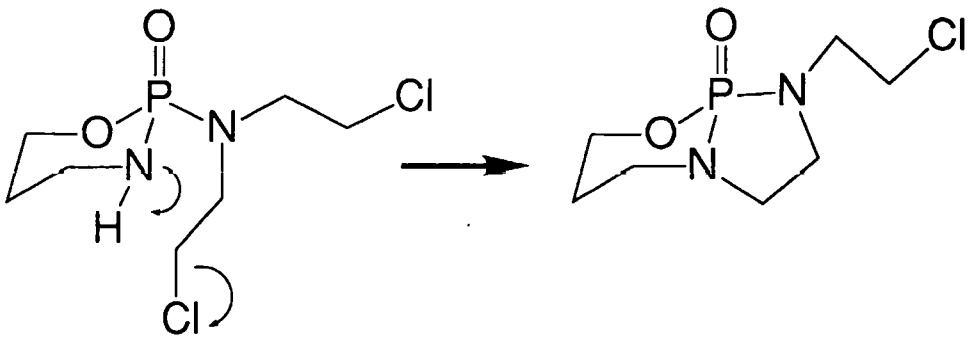
Fig. 14

Compound	*in vitro* Alkylating Activity %[52]	*in vitro* Relative Chloride ion Hydrolysis[53]	*in vivo* Therapeutic index[3] (LD50/CD50)
nor-Nitrogen Mustard (III)	100	1.00	4.4
Phosporamide Mustard (XXXVI)	90	4.00	3.5
Cyclophosphamide (XXXI)	1.3	700	175.0

Table 3

Therapeutic Index Comparison Between Selected Alkylating Agents

However, cyclophosphamide itself is found to undergo an essentially deactivating intramolecular cyclization[54-5] which renders the molecule virtually inert.



XXXI **XXXIX**

Fig. 15

Additionally, contradictory evidence is given in work by Struck *et al*[56] who noted that microsomally activated solutions[57] of cyclophosphamide are more toxic to L1210 leukaemia cells *in vitro* than equally alkylating solutions of phosphoramide mustard. These facts suggest that extracellularly generated phosphoramide mustard cannot account for all the anti-tumour effects of cyclophosphamide and could imply that other factors, such as the presence of the toxic acrolein, are involved in the cytotoxicity of cyclophosphamide.[42] (It should be noted that the comparison is made more complicated when bioavailabilty and other such physical variables of the drugs are considered).

The situation is further complicated by the release of nor-nitrogen mustard by the hydrolysis of phosphoramide mustard. Earlier reports[56] indicated that nor-nitrogen mustard (III) has a high alkylating ability according to the NBP test[58] but this work was carried out at pH 4.6. At physiological pH, *ie*: 7.4, nor-nitrogen mustard becomes deactivated by the loss of a proton from the aziridinium ion XL, giving the much more stable, and hence less reactive, aziridine (XLI).[27] See fig. 16.

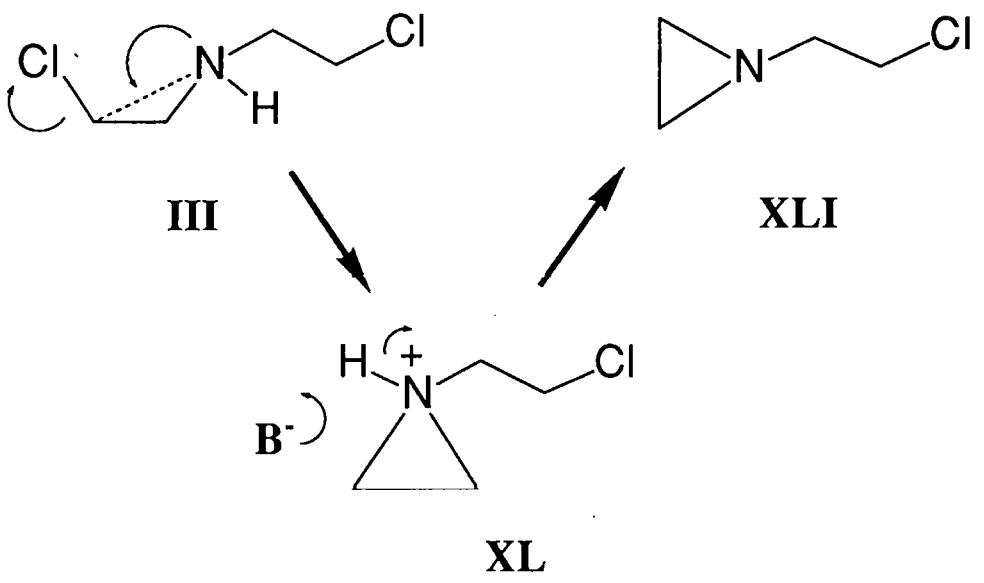


Fig. 16

Despite the uncertainties, it is widely believed that phosphoramide mustard is responsible for the major alkylating effects of the prodrug cyclophosphamide, and phosphoramide mustard is used extensively in studies of the mode of alkylation that gives the anti-cancer effects.

I.4.3 Mode of Alkylation Exhibited by Phosphoramide Mustard

An important contribution to this previously little understood area of chemistry came with the publication of work by Engle, Zon and Egan in 1982.[34]

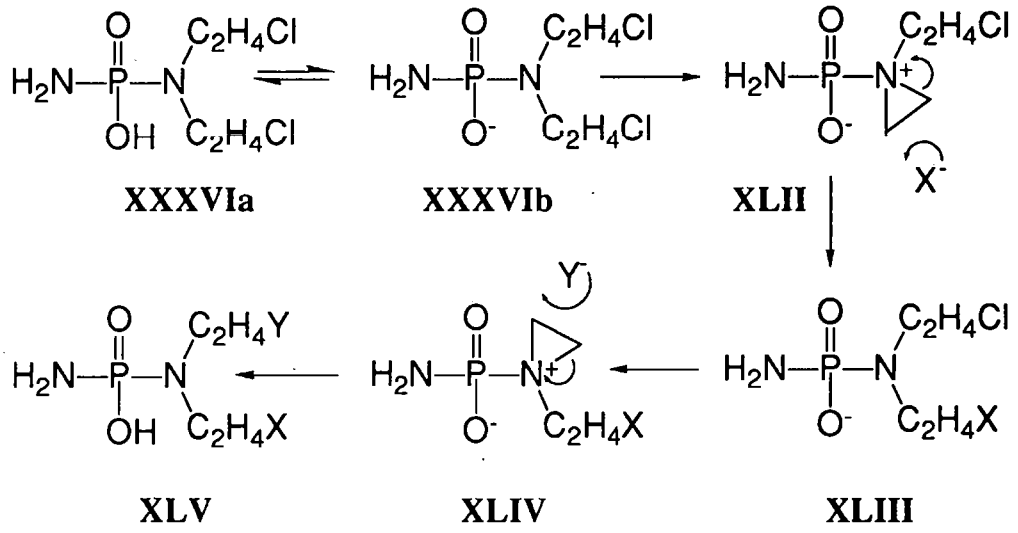
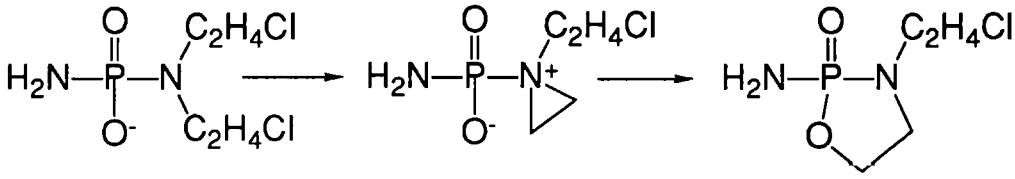


Fig. 17

Using ^{31}P Nuclear Magnetic Resonance (NMR) spectroscopy they were able to follow the hydrolysis, under varying pH, of phosphoramide mustard and

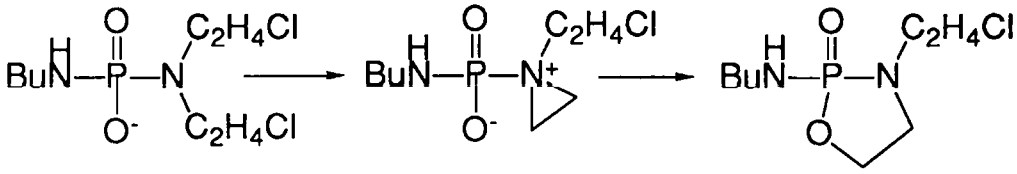
subsequently the effects brought about by varying the phosphoryl substituents. The hydrolysis of phosphoramide mustard is believed to follow a two step process for consecutive alkylation of each 2-chloroethyl group via an aziridinium ion intermediate. See fig. 17. Direct evidence for the presence of the aziridinium ion was seen in the ^{31}P NMR, which had a half-life that was dependent upon the concentration of hydroxyl ions thus precluding the possibility of the signal being due to XLIII which would not be expected to be pH dependent. The presence of the aziridinium ion intermediate was also confirmed by trapping the ion using sodium 2-mercaptoethylsulphonate (mesnum).[54] (Similar experiments with thiourea could not demonstrate this).[54, 59]



XXXVIb **XLII** **XLVI**

Fig. 18

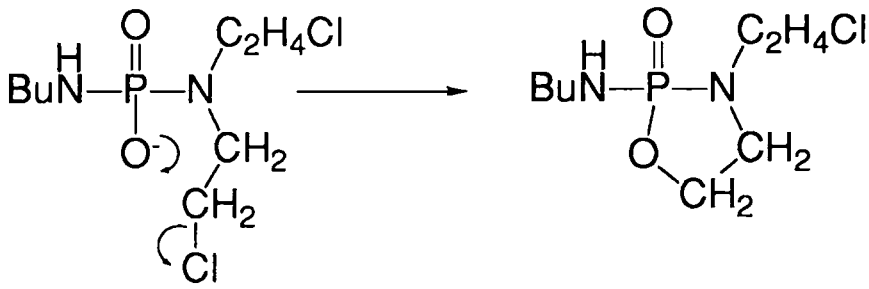
The importance of the substituents on the phosphorus atom was first noted by Friedman,[60] who attributed the lesser alkylating activity of ester derivatives of XXXVI to the loss in ability to form XXXVIb, and hence a stabilised aziridinium ion. An intramolecular*O*-alkylation of phosphoramide in acetone has also been seen[61] (see fig. 18) but interestingly, no intramolecular *N'*-alkylated product analogous to XXXIX in fig 15. Zon *et al*[34] also observed intra-molecular *O*-alkylation products in the *n*-butyl derivative of phosphoramide mustard, XLVII (fig. 19), by mass spectroscopic evidence.



XLVII **XLVIII** **XLIX**

Fig. 19

Evidence in the ^{31}P NMR was seen for the first aziridinium ion as before but it is not unreasonable to predict that XLVII could react directly with an S_N2 type mechanism as previously found with carbon dioxide and nor-nitrogen mustard (fig. 11). See fig. 20.

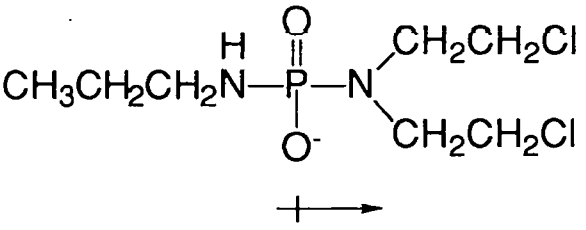


XLVII **XLIX**

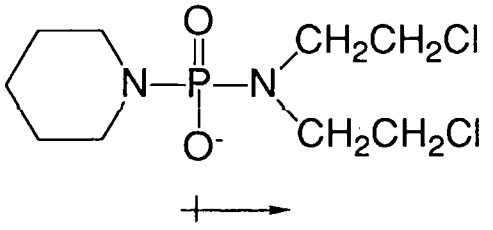
Fig. 20

This mechanism is supported by Zon's evidence that *N'*-substituted alkyl analogues, L and LI, enhance *O*-alkylation products, which can be explained

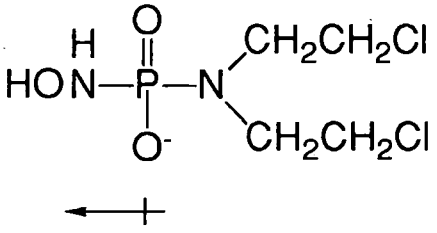
by the increased electron density on the oxygen. Likewise, oxygen N'-substituted compounds, LII, retard the rate of decomposition of phosphorylated mustards which could be due to a reduction in electron density on the oxygen. See fig. 21. If this is the mechanism, the need for the anchimeric effect from the mustard nitrogen's loan pair of electrons is avoided, as this is instead offered by the substituents on the phosphorus. Thus, it can be expected that a carbon mustard structure will be biologically active.



L $k = 46 \times 10^{-3}\ min^{-1}$



LI $k = 530 \times 10^{-3}\ min^{-1}$



LII $k = 0.63 \times 10^{-3}\ min^{-1}$

k = first order rate constant for the decomposition of substituted phosphoramides as determined by ^{31}P NMR spectroscopy[34].

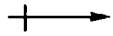
 = relative direction of dipole.

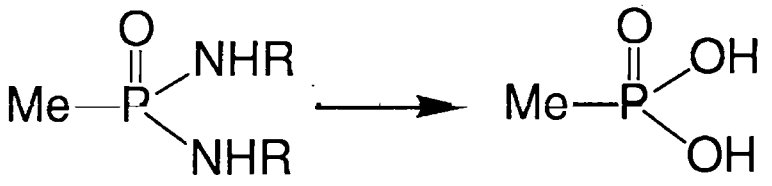
Fig. 21

I.5 Carbon Mustards

I.5.1 Why Carbon Mustards?

It has already been discussed (section I.4.4) that there may exist a mechanism whereby the anchimeric effect of the nitrogen atom of the mustard is not required. Therefore, it follows that the nitrogen could be replaced altogether with another atom which may not necessarily be able to offer any neighbouring group assistance: such an example is carbon.

What would the purpose of this substitution be? One of the major problems of the phosphorylated nitrogen mustard is the very highly acid-labile phosphorus-nitrogen (P-N) bond - a point which has often been overlooked in the literature. Apart from introducing synthetic problems (which in themselves can be quite considerable),[62] a bond as susceptible as this must lead to a considerable amount of uncertainty in the drug's metabolism *in vivo*. A phosphorus-carbon (P-C) bond is known to be less acid-labile than the equivalent P-N bond as neatly proved with the compound methyl phosphonicdiamide, LXIII (see fig. 22).



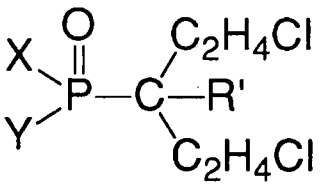
LXIII **LIV**

Fig. 22

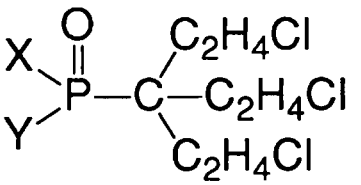
Upon contact with water, the amide moieties are quickly hydrolysed but the resulting methyl phosphonic acid, LIV, will remain in solution indefinitely. In Haddow's original requirements for a biologically active drug, the halogen atoms had to be of a certain minimum reactivity. Without the anchimeric effect of the mustard heteroatom, the enhanced reactivity has to originate from a phosphoryl substituent which, as discussed earlier, is feasible, at least in theory.

Another advantage offered by carbon mustards is the existence of an additional substituent. This could be used to include groups which could both activate the halogen atoms and increase the drug's variety of applications to suit specific targets. There is also the possibility of the extra substituent being a third 2-chloroethyl group, a tris-carbon mustard, which would be expected to increase the drug's cross-linking ability and hence its potency. See fig. 23.

Thus, the possibilities offered by phosphorylated carbon mustards as potential anti-cancer drugs are exciting. With this in mind, the project set out to synthesize and study a number of these compounds to assay their viability as anti-tumour agents. It is an important class of compounds which have had no mention in the literature and so their study is a necessary step to fill a gap in our knowledge of this vital area of chemistry.



LV



LVI

Fig. 23

I.5.2 Target Molecules

Because of the minimum reactivity shown by the phosphonate type compounds, it was planned to start with these initially to assemble the molecule, thus avoiding intramolecular reaction complications. Friedman and Seligman[38] had shown that it was possible to manipulate the phosphoryl substituents with the more delicate nitrogen mustards and so it was assumed that this could also be done with the carbon mustards.

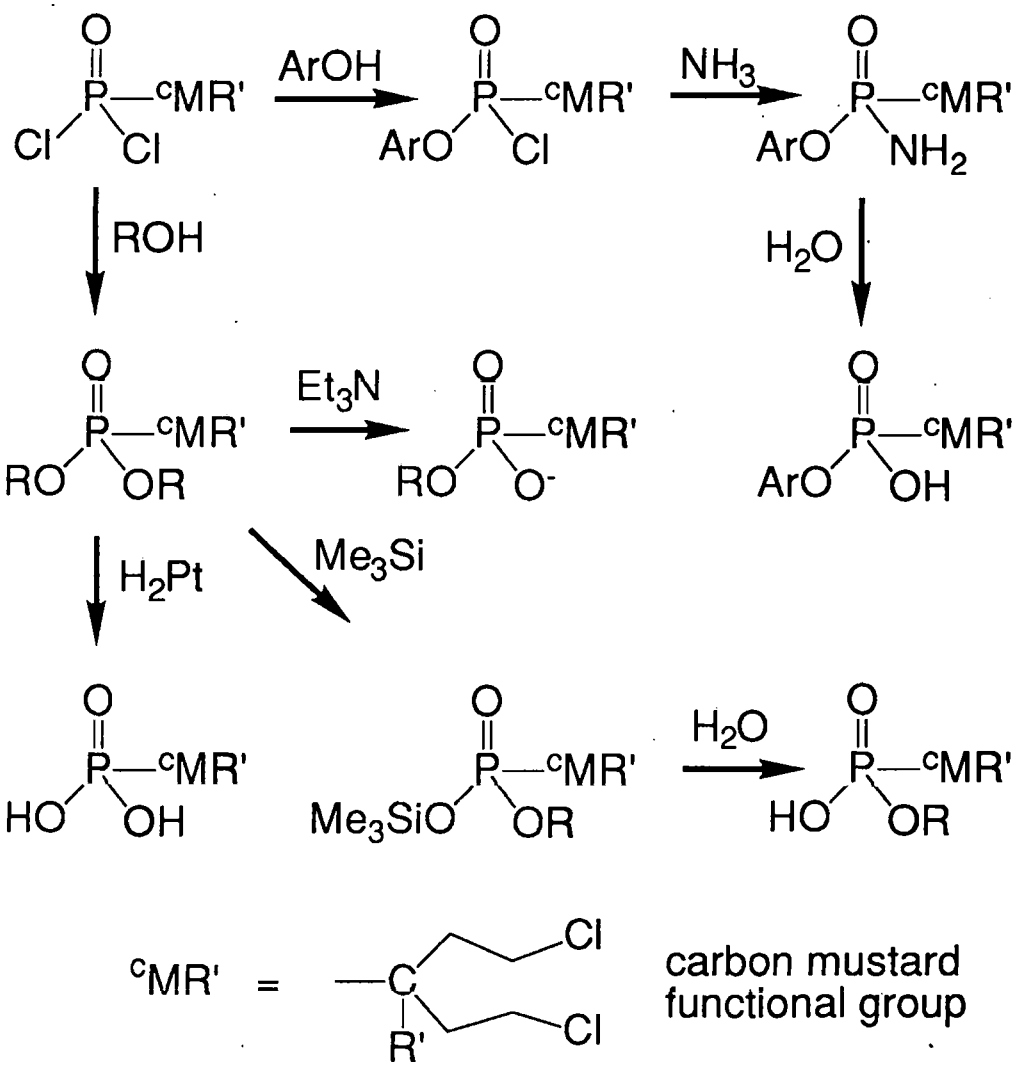
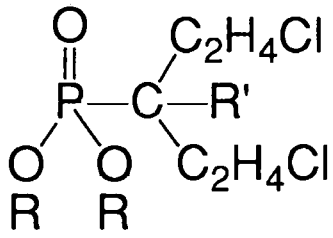
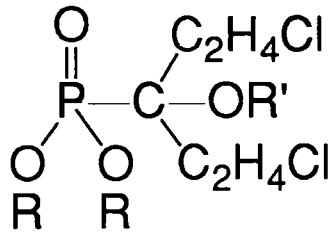


Fig. 24

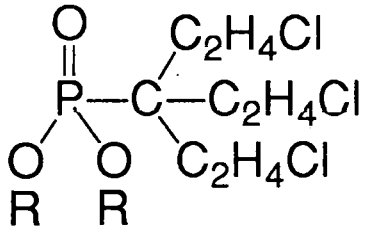
Fig. 24 illustrates the adapted manipulations that are expected to be possible on the carbon mustards. Hence, the phosphonates would be the first molecules to be made. Initially, the extra valency of the carbon mustard would be used to attach alkyl or aryl groups but later, when the synthetic details had been elucidated, it was planned to explore the possibilities of other functional substituents such as ethers, esters, amides, pyridines and even the additional 2-chloroethyl group as mentioned earlier. See fig 25.



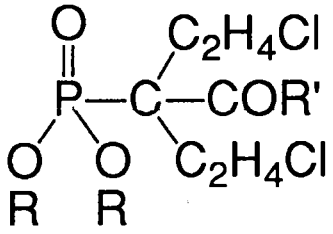
LVII



LVIII



LIX



LX

Fig. 25

This extra ability over the nitrogen mustards, to manipulate the molecule could prove to be a very powerful advantage of the carbon mustards.

I.5.3 Synthetic Strategy

The possible synthetic routes to the carbon mustard phosphonates are almost as diverse as phosphorus chemistry itself. A notable disadvantage with the

synthesis of carbon mustards, however, is the loss of nucleophilicity (*ie*: the exchange of a nitrogen atom for a carbon atom thus losing attack site specificity) which is important when forming the P-C bond. Many of the routes to the nitrogen mustards utilised the nitrogen's nucleophilicity to from the P-N bond.

The possible routes to the carbon mustards can be broadly grouped into three main categories:

(i) additions to the phosphonate skeleton;

(ii) condensation of the phosphoryl to mustard groups with phosphorus acting as an electrophile; and

(iii) condensation of the phosphoryl to mustard groups with phosphorus acting as a nucleophile.

These route each have their own particular advantages and disadvantages and these will be discussed separately below.

I.5.3.1 Additions to Phosphonate Skeleton

By starting with the desired phosphonate skeleton, addition of each 2-chloroethyl group by way of successive replacement of the moderately acidic α-hydrogens could theoretically give the desired carbon mustard phosphonate. See fig. 26.

The advantage of this route is that the initial phosphonates (LXI) are easily made from the Arbusov condensation of the corresponding alkyl phosphate

and haloalkane.[63] There would be relatively few steps to follow to produce the carbon mustard, either directly or by introducing latent 2-chloroethyl groups.

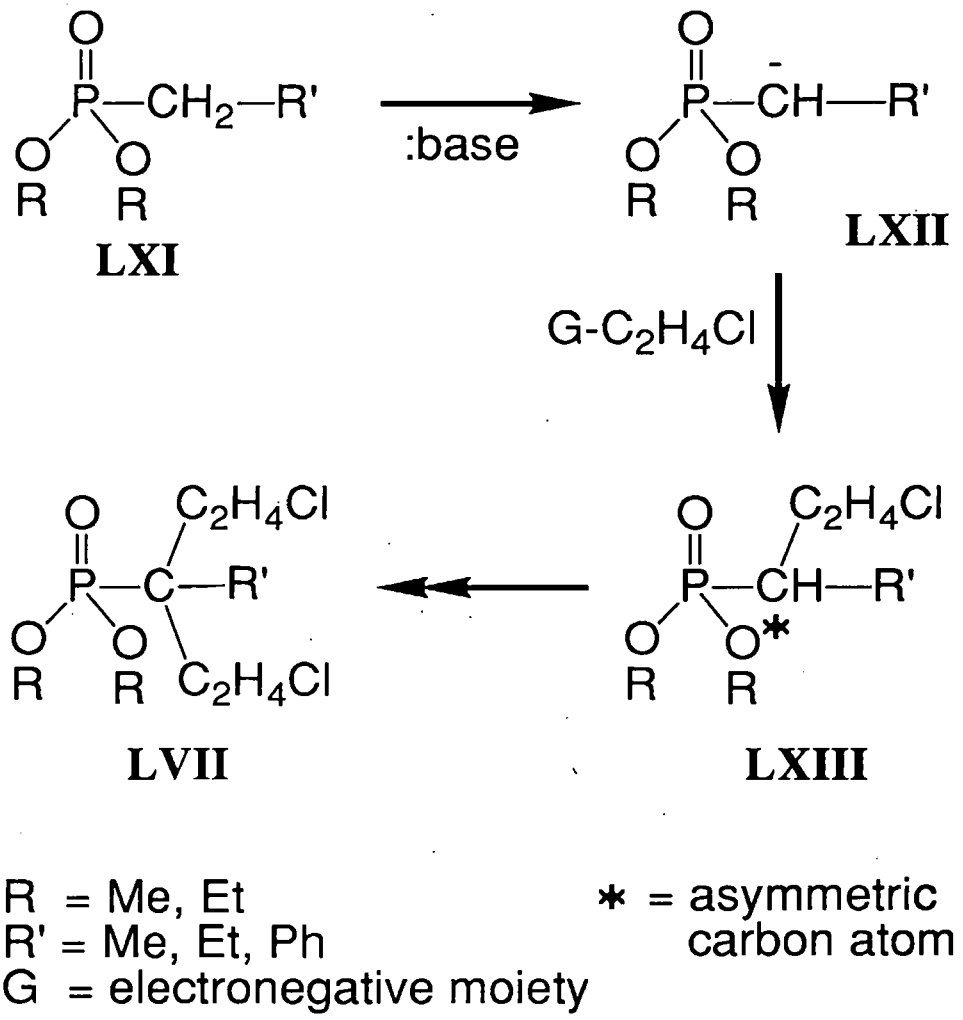


Fig. 26

I.5.3.2 Condensation of Phosphoryl and Mustard Groups with Electrophilic Phosphorus

Neutral phosphorus compounds can contain trivalent or pentavalent phosphorus. The latter is achieved by including the 3d electronic orbital in the molecular bonding orbital system.

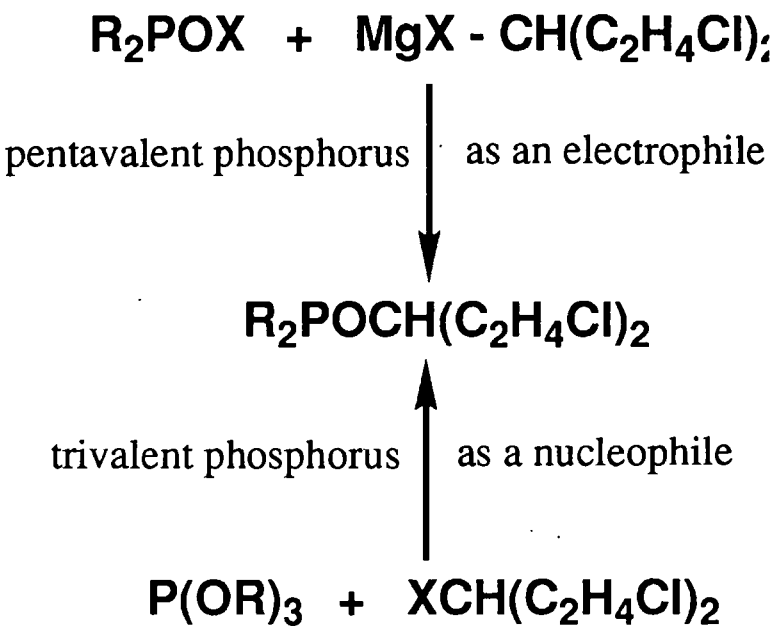


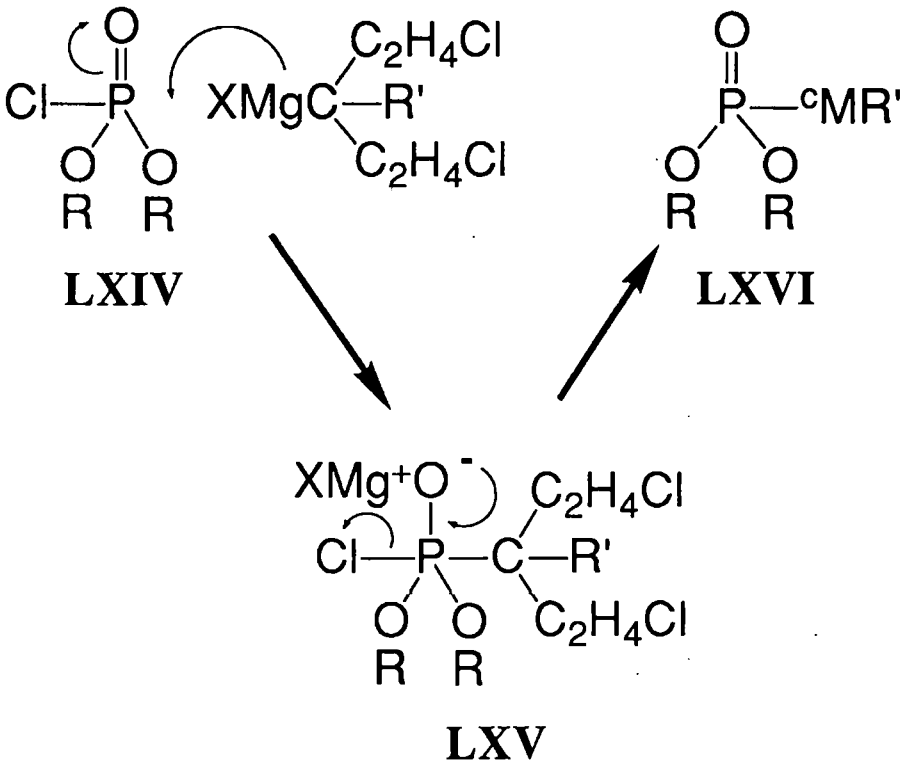
Fig. 27

The two states of phosphorus differ markedly in their electron affinity: trivalent phosphorus is similar to nitrogen in that it has a lone pair of electrons and hence, is relatively nucleophilic; pentavalent phosphorus, however, is notably electrophilic. See fig. 27.

Using either form of phosphorus to achieve the condensation with the carbon mustard is quite different from the previous strategy as here the phosphorus is attached in the last step to a previously assembled, substituted 2-chloroethyl moiety. This method has the advantage that the chemistry of synthesis does not involve phosphorus until the last step and so would be expected to be relatively conventional and the products easily purified. Disadvantages include the associated problems of a long linear synthesis and the problems of the last step which involves manipulation of a moderately bulky molecule.

For effective electrophilic phosphorus attack, an anionic centre must be developed on the complementary molecule. With nitrogen mustards this

presented little problem as the nitrogen's lone pair of electrons provide an ideal such site. With carbon mustards, however, this must be induced by the use of substituents and for this purpose it can be expected that Grignard reagents and other organometallic compounds will be of importance. Indeed, penta-valent dialkyl chlorophosphates do react with Grignard reagents providing a useful method for forming P-C bonds.[64] See fig. 28.



$^{C}MR'$ = Substituted carbon mustard moiety

Fig 28

I.5.3.3 Condensation of Phosphoryl and Mustard Groups with Nucleophilic Phosphorus

One of the most common and versatile methods for forming a P-C bond is by way of the Arbusov reaction. The nucleophilicity of trivalent phosphorus is

combined with an alkyl halide's polarised bond and good leaving group qualities to provide a clean reaction and usually with high yield. The general form of this reaction is illustrated in fig. 29.

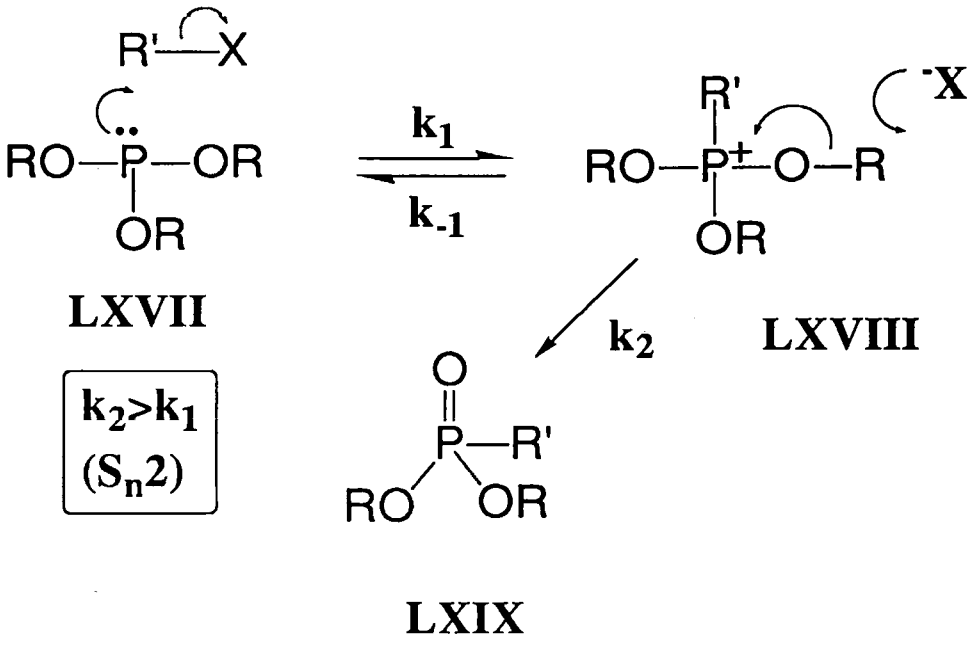


Fig. 29
The Arbusov Reaction

It is feasible, steric hindrance permitting, that a tertiary halide mustard precursor, such as LXXII, could be used together with a suitable phosphite (but triphenyl phosphite blocks the Arbusov reaction) to give the desired compound by way of nucleophilic attack on the phosphorus.

There are many variations to this reaction. For example, the phosphorus may attack as a previously formed anion by treatment of a dialkyl phosphite with *n*-butyllithium. This will have the effect of increasing the nucleophilicity of the trialkyl phosphite. See fig. 30.

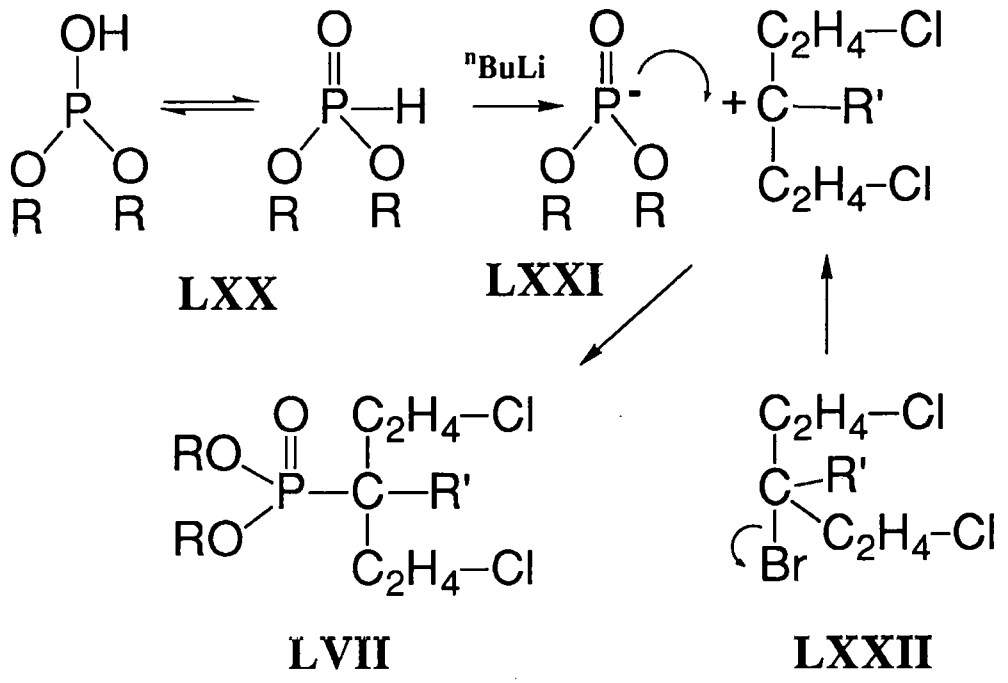


Fig. 30

It is even possible that the cation of LXXII may be previously formed with exposure to silver perchlorate or another poorly nucleophilic anionic species. This might have the effect of accelerating the rate determining step which is the formation of the tertiary carbonium ion. See fig. 31.

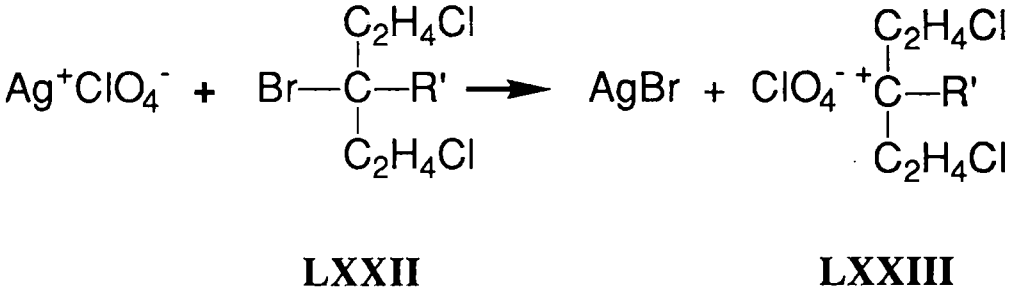


Fig. 31

Compounds of the form of LVIII (fig. 25) might also be made utilising the nucleophilicity of suitable phosphorus compounds. Anionic species, LXXI

can be expected to attack 1,5-dichloropentan-3-one (LXXIV) to give the alcohol LXXVI, which can then be acylated or alkylated to give compounds of possible biological interest.[64] See fig. 32.

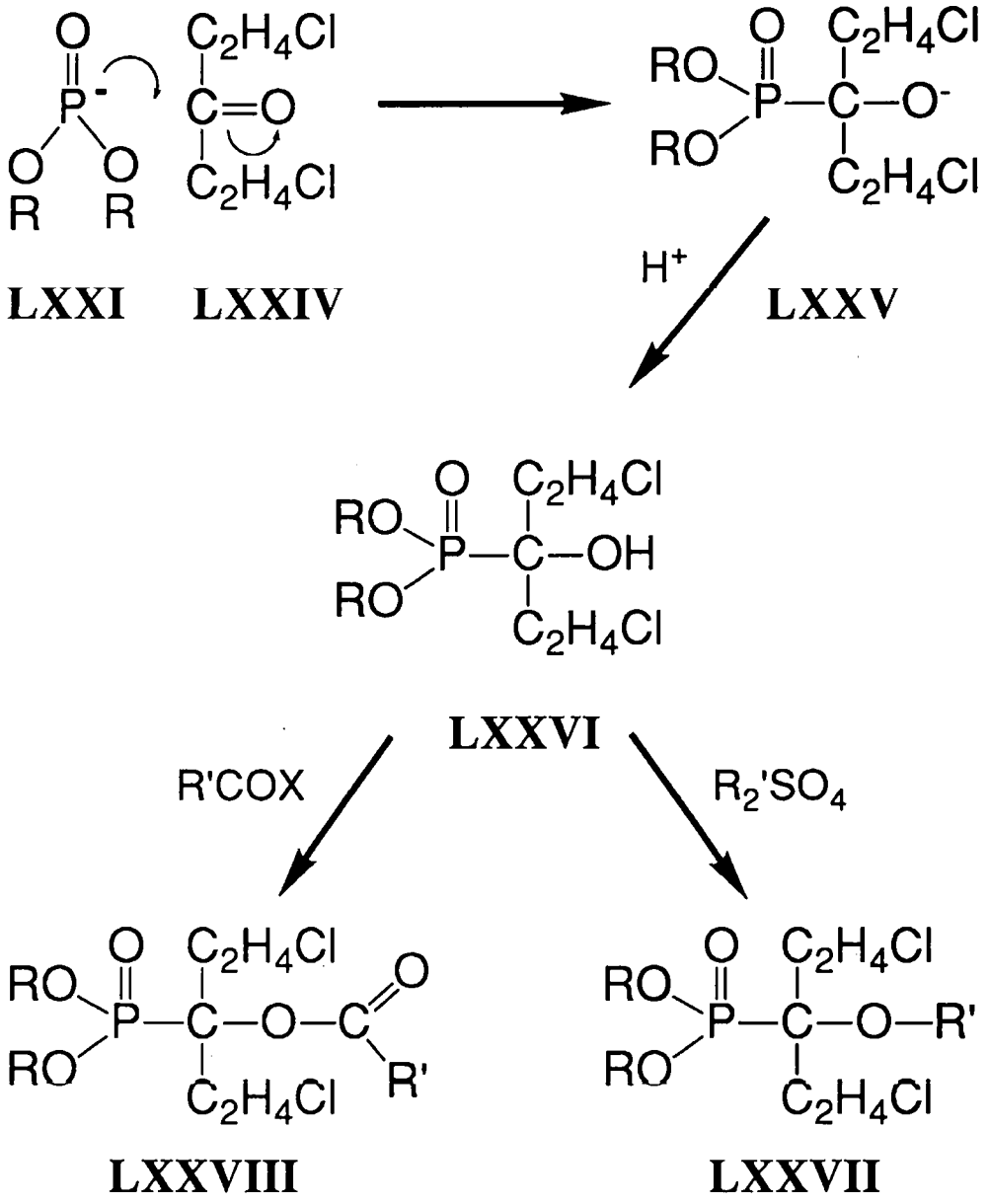


Fig. 32

For comparisons in expected kinetic studies on these compounds it is useful to synthesize the mono-functional analogue LXXXI which, however, will

probably be biologically inactive as discussed above in section I.1.2. See fig. 33.

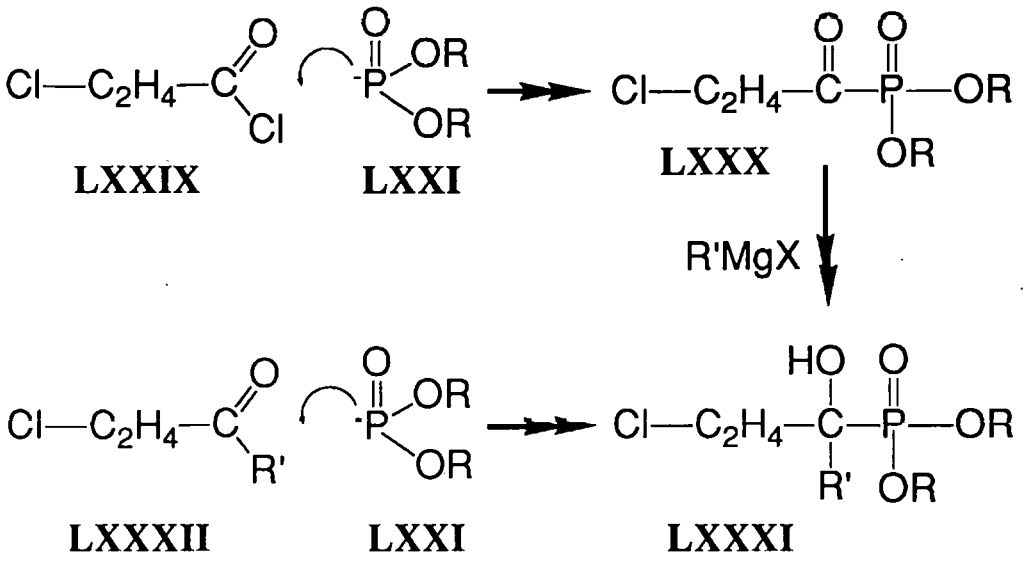


Fig. 33

Although these compounds have little in common with previously made phosphorylated nitrogen mustards, limited analogous synthetic chemistry does exist.

One of the most promising and potentially useful synthesis is an adapted scheme used by Foucaud[65] which utilises potassium (or caesium) fluoride to abstract a proton from a dialkyl phosphite to form the phosphoryl anion (LXXI). This reaction produces much cleaner products which are more easily purified.

Despite this, and the general diversity of all these synthetic routes, at the close of this project none of the desired compounds has been made. The various reasons for these failures will be discussed in Part II.

I.5.4 Tri-functional Carbon Mustards

With carbon mustards there exists the possibility of introducing a third 2-chloroethyl group. As mentioned earlier, the biological activity of the mustards is believed to be due to their ability to cross-link various nuclear elements within the cell and so increasing the valency for alkylation could be expected to increase the cross-linking ability.

This would be expected for two reasons. First, the local concentration of 2-chloroethyl groups has effectively been increased by 50% and so an increased rate of reaction can be expected. And secondly, if a comparison is made to the binding of multivalent antibodies, such as IgG or IgA, the increase in reaction rate is likely to be far greater than that expected by a simple linear relationship to the number of binding arms.[66] This is due to a facilatative action, by holding the molecule in place by one arm giving it an increased time in the environment of the substrate for the second arm to react. Thus, a tri-functional mustard, of which only the tris-2-chloroethylamine is allowed with the nitrogen series (and which has no anchimeric assistance),[16] is theoretically of much greater interest.

However, the synthesis of a tris-(2-chloroethyl)methane moiety raises considerable problems. As with the bi-functional carbon mustards, two schemes may be feasible: the first involves additions to a phosphonate skeleton, and the other, the condensation of mustard and phosphoryl moieties. Both schemes will, however, need some degree of protection to the chloride groups to avoid β-elimination. They are both also of some length which with the experience of the similar chemistry, may not be a minor problem. See fig. 34.

The routes to the tri-functional carbon mustards was not tried in this project, however, as synthesis of the bi-functional mustards was not completed.

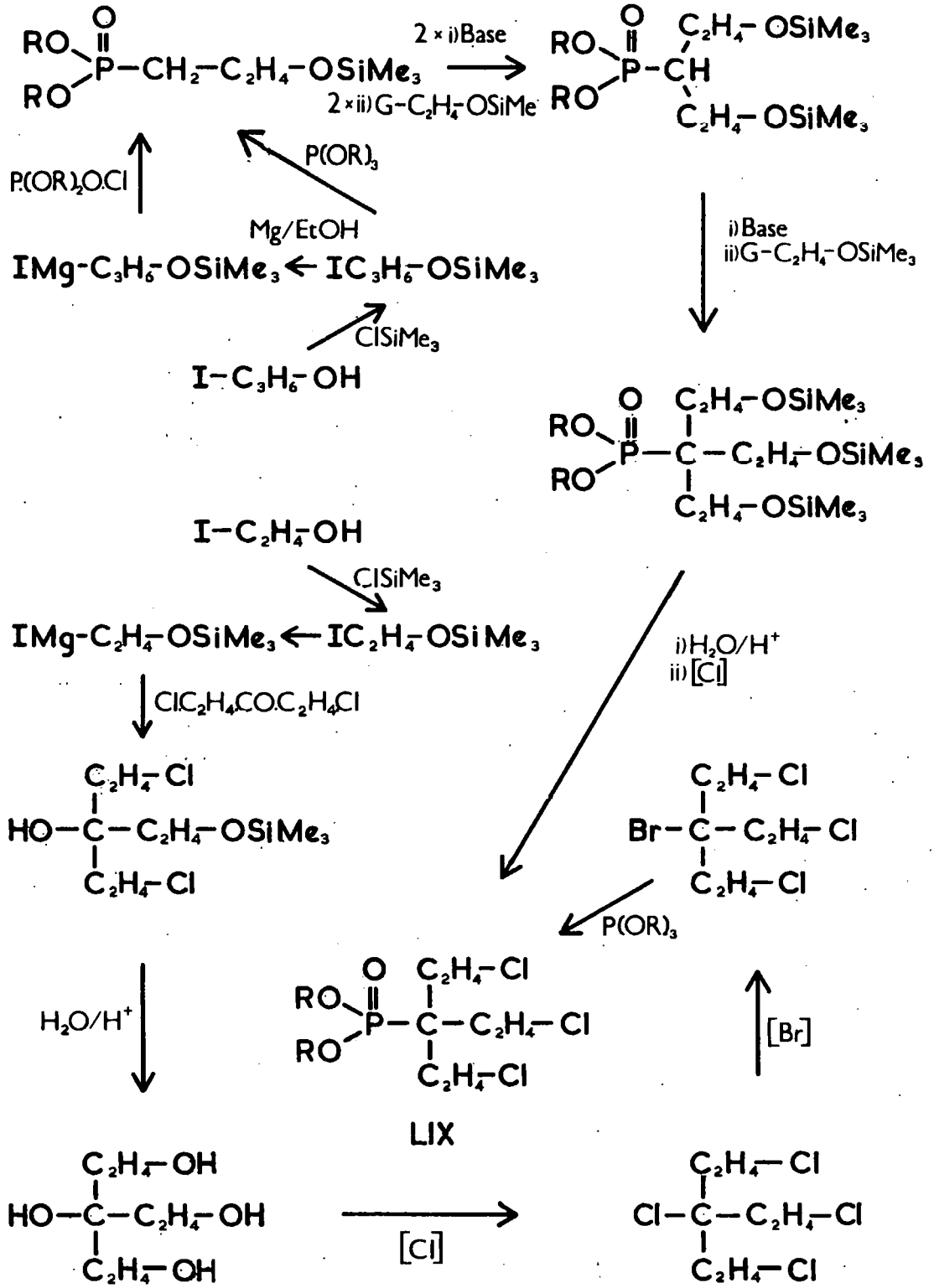


Fig. 34

Possible synthetic pathways to tri-functional carbon mustards.

I.5.5 Carbonyl Containing Carbon Mustards

While the most successful anti-tumour mustards have been the phosphorylated derivatives of nitrogen mustards, this is not a prerequisit for therapuetically useful nitrogen mustard drugs (*eg:* chlorambucil - see fig. 12). Similarly, carbon mustards without a phosphryl group are feasible and would, at least, provide useful kinetic references to the phosphorylated carbon mustards. A proposed route to their synthesis, as shown in fig. 35, would give the non-substituted products via decarboxylation of a substituted malonate ester.

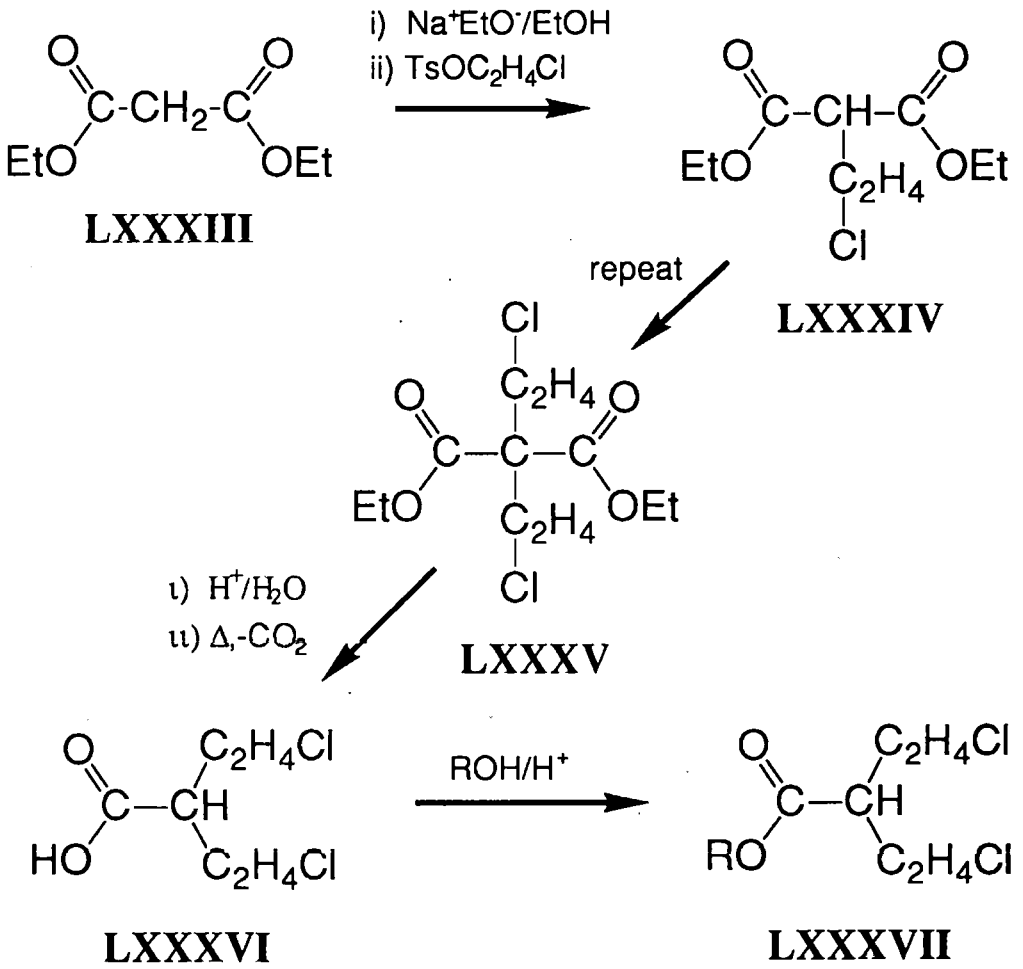


Fig. 35

The hydrogen derivative, or non-substituted derivative, of the phosphoryl carbon mustards was deliberately avoided, in the first instance because of the possible spontaneous intramolecular alkylation. The carbonyl ester, however, would not be expected to activate the 2-chloroethyl groups to the same extent and so these compounds would be expected to be stable.

Part II: Results & Discussion

Results & Discussion

II.1 Introduction

This section describes the methods pursued in the attempted synthesis of the phosphoryl carbon mustards. The approach is grouped into two broad categories:

(i) the addition of a carbon mustard moiety to a phosphoryl group; and

(ii) the progressive assembly of the mustard on an appropriate phosphoryl skeleton.

The experimental handling of phosphorus containing compounds tends to be hindered by the difficulty in separating reaction products. The completion of the carbon mustard moiety's formation before condensation to the phosphoryl group not only avoids this difficulty until the last step but also allows the majority of experimental work to be undertaken on relatively small molecules. Addition of the phosphorus group to the carbon mustard may be accomplished by either nucleophilic or electrophilic attack by the phosphorus component on the mustard, see fig. 27. (Sections II.2.2 and II.2.3 address these approaches respectively).

However, the late conjugation of the phosphorus moiety requires an attack on a sterically hindered carbon atom and this proved to be a difficult problem. This, of course, is not a problem with the progressive assembly of the phosphoryl mustard but, apart from the experimental difficulties already mentioned, the susceptibility of the 2-chloroethyl groups to undergo β-

elimination as the phosphorus compounds are manipulated prevents many of the useful synthetic reactions.

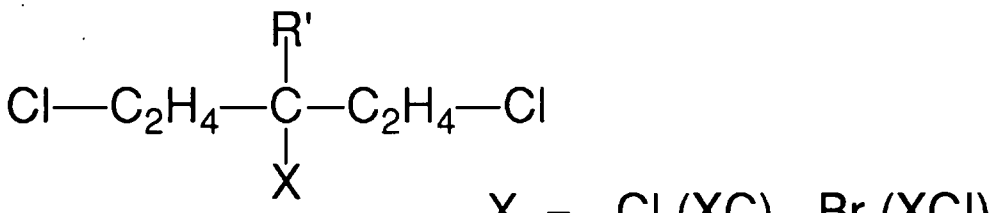
The strategy involving the conjugation of a previously synthesised carbon mustard with the phosphoryl group will be discussed first and in this section the synthesis of phosphoryl compounds with only one 2-chloroethyl group will also be included. The synthesis of carboxylate carbon mustards, however, will come under the strategy involving the progressive assembly of the phosphoryl mustard and is discussed in section II.3.

II.2 The Condensation of Mustard and Phosphoryl Moieties

Before condensation of the phosphoryl component is made to the carbon mustard, the latter has to be synthesised and the methods and results of this synthesis are discussed in the next section.

II.2.1 Synthesis of the Substituted Carbon Mustard

The type of compound being sought has the general formula given in fig. 36. Remarkably, no evidence for the general compound LXXXVIII (see fig. 36) has been found in the literature.[75] However, this is an important compound in this work and is included in many of the synthetic strategies for the phosphorylated carbon mustards, both by nucleophilic and electrophilic attack.



LXXXVIII

Fig. 36

There are a number of feasible routes to its synthesis but the one which was used involves the acylation of 3-chloropropionylchloride with ethylene to give

1,5-dichloropentan-3-one, to which the substituent was added with a Grignard reagent and finally, halogenation of the alcohol. Its synthesis is outlined in fig. 37, and is discussed in the next section.

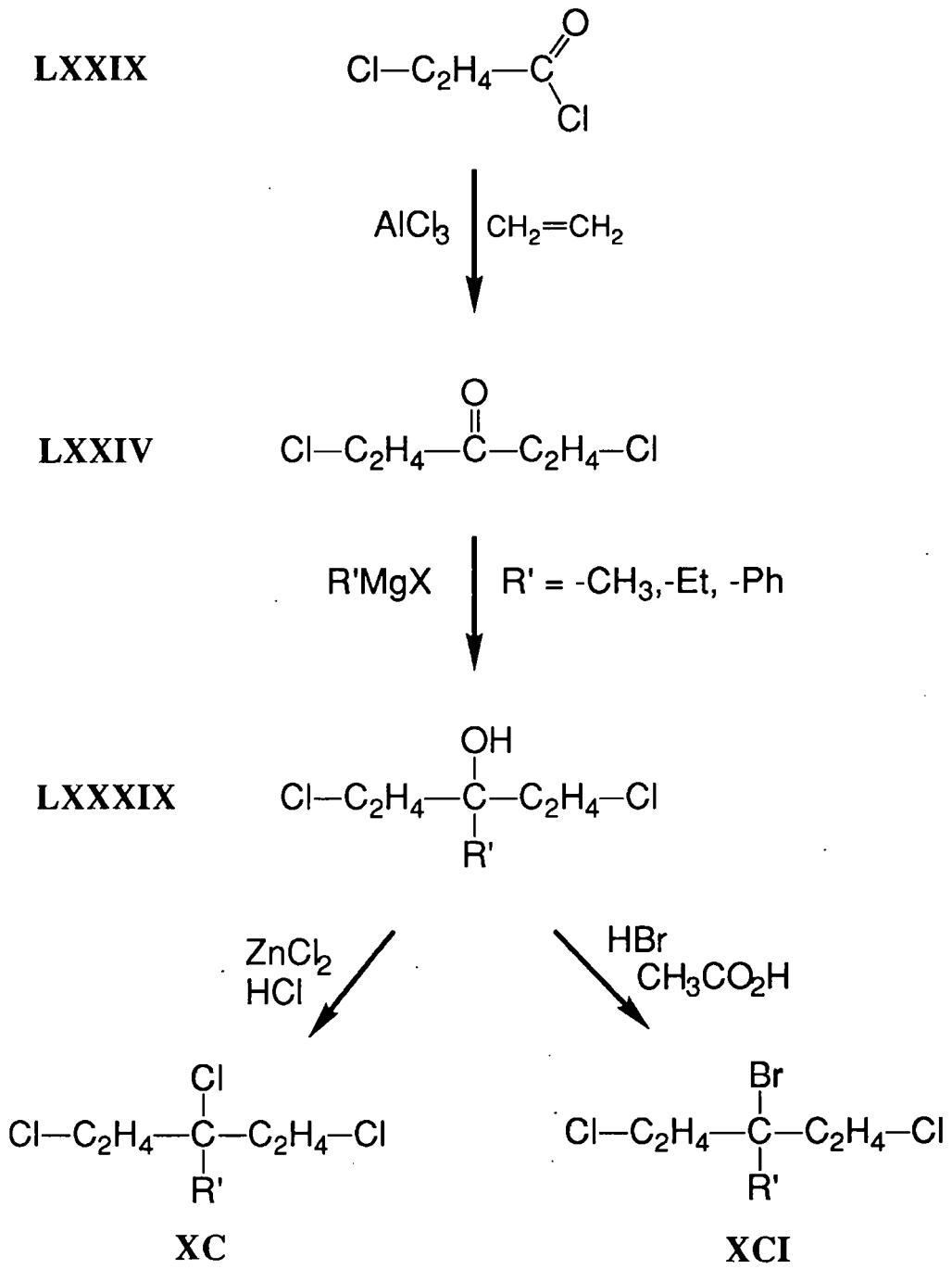


Fig. 37

Synthesis of Halo-Carbon Mustards

II.2.1.1 Synthesis of 1,5-dichloropentan-3-one

The first step in the synthesis requires the formation of 1,5-dichloropentan-3-one. A modification[67] of the synthesis reported by Catch *et al*[68] was used to obtain 1,5-dichloropentan-3-one (LXXIV) from 3-chloropropionyl chloride (LXXIX) although the stoichiometric yields reported could not be achieved (a 3.4 mole scale gave a yield of 78%). The ketone (LXXIV) was recognised by the shift to higher field of the α-methylene protons (α to the carbonyl group, that is) from δ3.35 (t, 4H, $^3J_{H,H}$ = 6Hz) in 3-chloropropionyl chloride to δ2.95 (t, 4H, $^3J_{H,H}$ = 6Hz) in the ketone.

The pure ketone was found to become contaminated within days, even when kept under dry nitrogen. To avoid this, after the methylene chloride used in purification of the ketone had been removed under reduced pressure, the ketone was immediately dissolved in freshly distilled, dried tetrahydrofuran to give a solution of known concentration which enabled future use without first removing the solvent. This was found to prevent decomposition and allow indefinite storage of the ketone.

The electronic environment of the methylene protons can be described by the degree of shielding that they are found to have in the ^{1}H NMR. For a saturated alkyl system a methylene proton might be expected to have a chemical shift of around δ1.25. In fact the α-methylene protons were found to have a chemical shift of δ2.95 and the β-methylene protons a value of δ3.80. This indicates that there is considerable deshielding in the local electronic environment of the methylene groups - especially for the β-methylene protons.

The modified Shoolery rules predict[69] a resonance at δ2.90 for the α-methylene protons and δ3.20 for the β-methylene protons. This is a good approximation for the α-methylene protons but the β-methylene protons

resonate a further 0.6ppm downfield indicating that they are even more deshielded than the already moderately deshielded α-methylene protons.

The inference that can be drawn from this is that the α-protons will be particularly susceptible to attack by a base. In addition the stability of any α-carbanion formed might be increased and so with the acidity mentioned above gives an explanation to the ease of the decay of the 2-chloroethyl group towards base induced β-elimination - a decomposition so often encountered with this work.

II.2.1.2 Synthesis of Substituted Carbon Mustard Alcohols

The substituent of the mustard, R', (R'= methyl, ethyl and phenyl) was introduced with a Grignard reagent to 1,5-dichloropentan-3-one, to give the carbon mustard alcohol (LXXXIX). The non-substituted alcohol (LXXXIX, R'=H) was avoided (initially, at least) as it was envisaged that the phosphorylated mustard would be highly susceptible to the loss of a proton and subsequent inactivation by the formation of a cyclopropane system similar to the aziridine formation seen with the nitrogen mustards. See fig. 16 & section I.4.2.

II.2.1.2.1 Identification of carbon mustard alcohols

The formation of the alcohol was confirmed by the ^1H NMR spectrum which, for R' = methyl, showed an upfield shift of the α-methylene protons to δ2.00 (t, 4H, $^3J_{H,H}$=6Hz) and the addition of a methyl signal at δ1.25 (s, 3H). The β-methylene protons give a well defined triplet at δ3.70 (t, 4H, $^3J_{H,H}$=6Hz) - a shift upfield of only 0.1ppm. The ethyl derivative (LXXXIX, R' = ethyl) gave

a similar upfield shift of the α-methylene protons and an ethyl signal at δ0.95 (t, 3H, $^3J_{H,H}$=5Hz) and δ1.55 (q, 2H, $^3J_{H,H}$=5Hz). The alcohol peak was found between δ2.0 - δ 4.0, was sharp and could usually be removed by a prolonged D_2O shake.

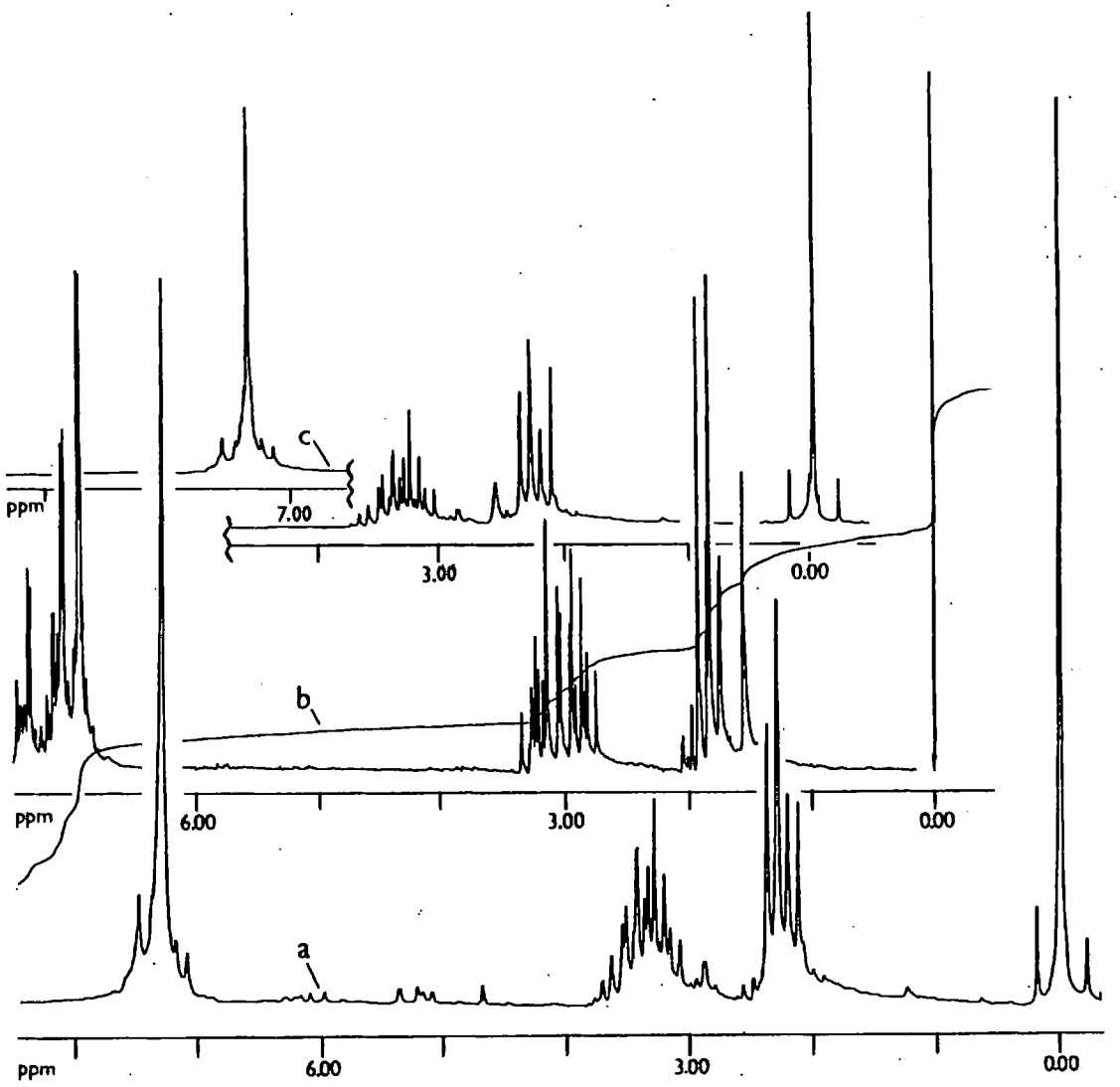


Fig. 38

^{1}H NMR 90MHz spectra of 1,5-dichloro-3-phenylpentan-3-ol. *Spectrum a:* solvent, $CDCl_3$ with D_2O; *Spectrum b*: solvent, C_6D_6 with D_2O; *Spectrum c:* solvent,$CDCl_3$ (without D_2O)

However, the phenyl derivative (LXXXIX, R' = phenyl) gave an unexpected ^{1}H NMR spectrum: while the α-methylene protons gave an expected triplet at δ2.30 (t, 4H), the β-methylene protons gave a broad multiplet centred around

δ3.40 (m, 4H). See fig. 38, spectrum c. The product was confirmed as LXXXIX, R' = phenyl, with ^{13}C NMR and mass spectrometry. The ^{13}C NMR gave a spectrum with three major aromatic tertiary carbon resonances between δ125 and δ128 and two aliphatic resonances due to the methylene carbons at δ40 and δ46 (compared to δ40.0 & δ44.6 seen with LXXXIX, R' = methyl) which became triplets in the off resonance spectrum. The tertiary aliphatic carbon resonance was seen within the deuterochloroform signal. The mass spectrum demonstrated the 2-chloroethyl cation (m/e 63,65) and the 3-chloro-1-hydroxy-1-phenyl-1-propyl cation (m/e 169,171) which were easily identified by the 3:1 ratio of peak intensities due to the mixture of chlorine isotopes. Tropylium and benzoyl cations were also seen in high abundances at m/e 91 and 105 respectively.

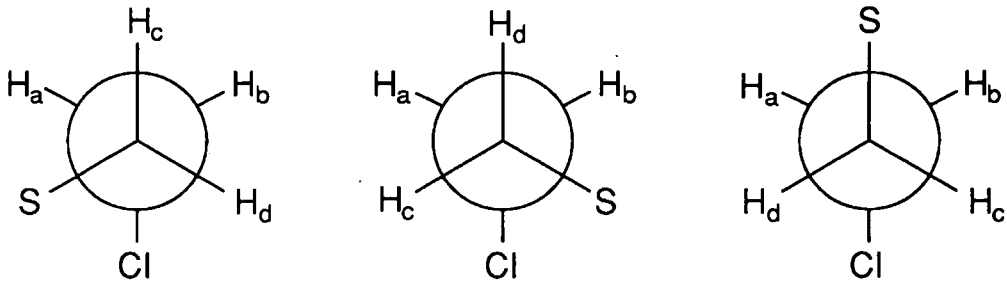
The integration of the multiplet in the ^1H NMR (spectrum b, fig. 38) is correct, indicating the complex to be the result of four protons. Assimilating the evidence, there is little doubt that LXXXIX, R' = -Ph had been made and that the multiplet seen at δ3.40 was due to the β-methylene protons. However, the reason for the broad multiplet seen in the ^1H NMR is difficult to establish, but a few ideas are now discussed.

With comparison to the methyl and ethyl derivatives, in which there is no multiplet seen in the NMR, it is clear that the phenyl substituent is a cause, either directly or indirectly, of the morphology of the resonance at δ3.40.

To consider this further it is useful to compare the spectra obtained for 1,5-dichloro-3-phenylpentan-3-ol and the parent compound, 3-phenylpentan-3-ol. The latter gives a simple A_2X_3 quartet for the methylene protons at δ1.85 (q, 4H, $^3J_{H,H}$=6Hz) and a triplet at δ0.75 (t , 6H, $^3J_{H,H}$=6Hz). Hence the phenyl ring *and* the chlorines are both necessary to resolve the magnetic non-

equivalence of the β-methylene protons and change the triplet into the observed multiplet.

Restricted rotation about the C_α-C_β bond is a possible reason for the developed magnetic inequivalence. When the gauche conformations of LXXXIX, R' = phenyl are considered as Newman projections along the C_α-C_β bond (see fig. 39), it can be seen that the two β-methylene protons are indeed not magnetically equivalent.



1 **2** **3**

S = $CPhR'C_2H_4Cl$

$H_a(3) = H_b(3)$
$H_a(2) = H_b(1)$
$H_b(2) = H_a(1)$

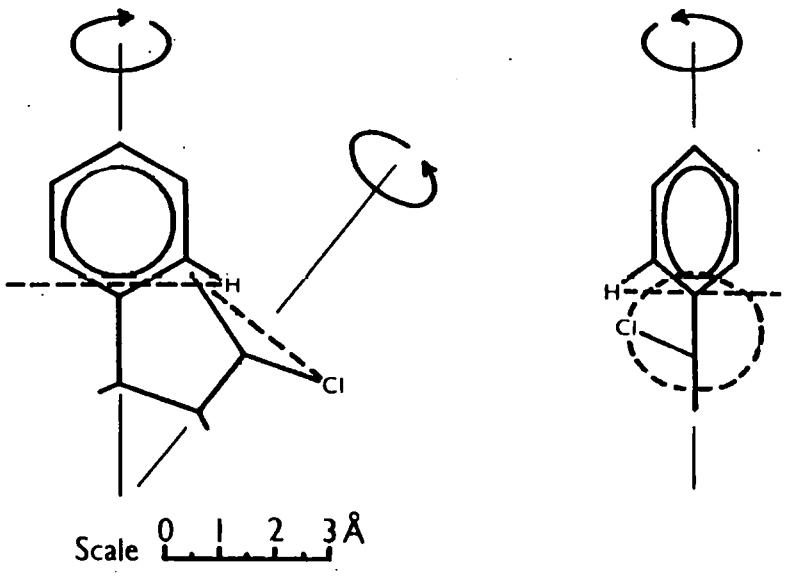
$H_a(1) \neq H_b(1)$
$H_a(2) \neq H_b(2)$

Fig. 39

Newman Projections of the gauche conformations of LXXXIX, R' = -Ph demonstrating β -methylene proton magnetic non-equivalence.

However, under normal circumstances, the high speed of rotation about the C_α-C_β bond, with respect to the time scale of the NMR spectrum, would be expected to average the different resonances due to their different

environments. One possible slowing factor for rotation might be that the bulky chlorine atom interacts with the ortho-protons of the phenyl group. See fig. 40.



LXXXIX, R′= –Ph

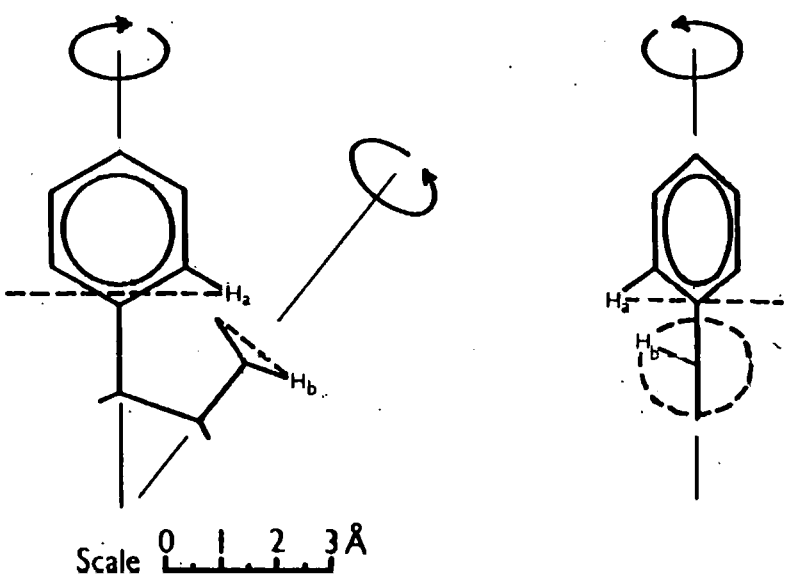
Fig. 40

If restricted rotation was an important feature, and first order splitting was observed, there would be a singlet and two doublets, probably with a non-intergral ratios to their areas (cf: ^{19}F spectrum of $CBrF_2CBr_2CN$ at -98°C).[70] This pattern is expected because in conformations 1 and 2 of figure 39, H_a and H_b are in non-identical magnetic environments. However, as $H_a(1)$ is equivalent to $H_b(2)$ and $H_a(2)$ is equivalent to $H_b(1)$, only two distinct proton environments exist. These would be expected to be seen as doublets in the NMR as for each conformation they are non-equivalent and so would couple with each other. Conformation 3 gives identical magnetic environments for

H_a and H_b and so only one resonance would be expected from this conformation.

This pattern of two doublets and one singlet would be expected to be further split into triplets by the α-methylene protons, ie: a maximum of 15 peaks would be seen if first order splitting was obeyed. As fig. 38 shows, this splitting morphology is not recognisable and while second order splitting could account for this, if this is an observed effect, this can only remain speculative.

An additional reason for magnetic non-equivalence of the β-methylene protons might be from the fact that the α-methylene protons, being attached to a prochiral centre, are diastereotopic. However, the simple triplets observed for the α- and β-methylene protons in the methyl and ethyl derivatives indicate that the methylene proton environments are not always sufficiently



LXXXIX, R′=-Ph

Fig. 41

different for this diastereotopicity to be observed. Of course, rotation does not influence the number of signals seen in the NMR for diastereotopic protons but again, as the multiplet is not seen in the compounds without both the chlorines and the phenyl ring, these substituents must play a part in exaggerating the diastereotopicity of the β-methylene protons should the effect exist.

From fig. 41 it can be seen that the β-methylene protons, in certain conformations, do come close to the *ortho*-protons of the phenyl ring and indeed the strong induced field of the benzene ring itself. And while restricted rotation would not be expected to produce an exaggerated diastereotopic effect, the chlorine, being much bulkier than the hydrogens, might be expected to hold the β-methylene protons in the influence of the ring for longer than without.

Other derivatives which were made include isopropyl, *n*-butyl and *t*-butyl but as these compounds were expected to offer greater steric hindrance and a less stable intermediate carbonium ion, their use was kept in second line to the other derivatives.

II.2.1.2.2 Potential Grignard reaction impurities

Although the desired alcohol was made, it is worth considering the identification of the side-products as this throws some light on to possible mechanisms of reactions discussed later in this work.

Reaction with the Grignard reagent gave poor yields (typically less than 40%) with the production of a large quantity of unidentified inert material. There are three possible sources for these impurities, alkene formation following the

loss of hydrogen chloride, polymerisation of the various alkenes, and hydrolysis of the 2-chloroethyl group during the work up of the Grignard reagent. All these side-products can be formed from either the starting ketone, LXXIV, or the desired alcohol end product, XXXIX, or even from a mixture of the two.

The starting ketone, LXXIV, is particularly susceptible to β-elimination. This is kinetically favourable due to the acidity of the α-hydrogens and thermodynamically favourable because of the formation of a conjugated product. See fig. 42.

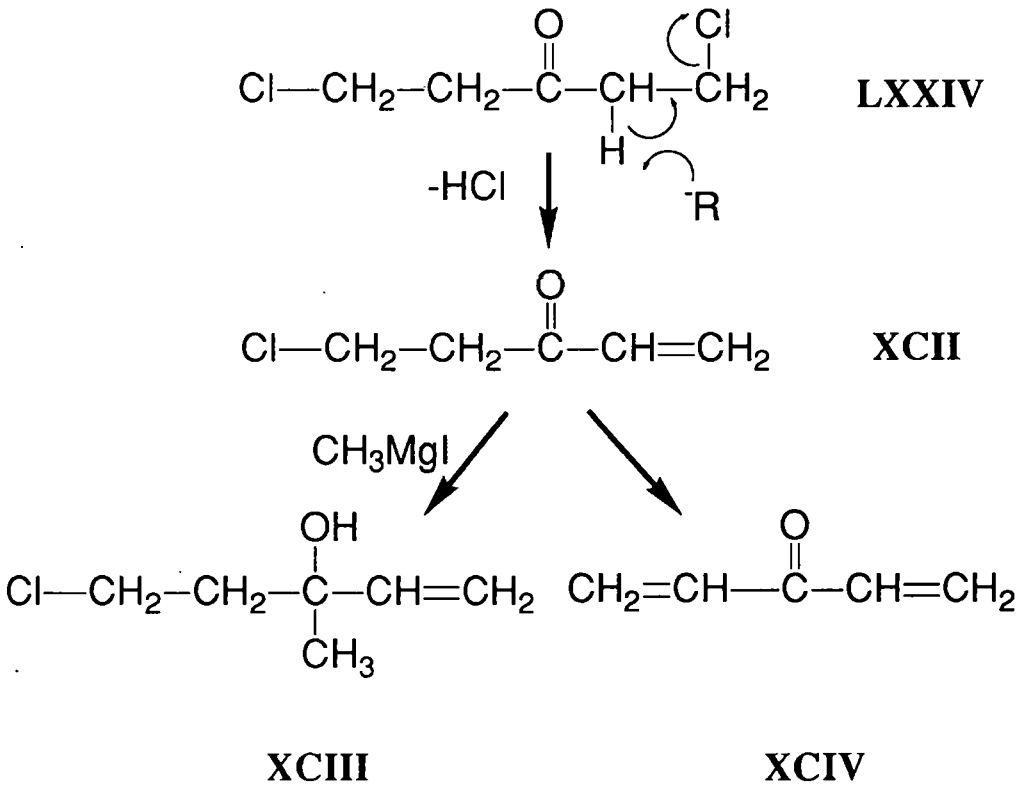


Fig. 42

The subsequent loss of another molecule of hydrogen chloride from the α,β-unsaturated ketone is possible but the product, pent-1,4-dien-3-one (XCIV)

does not have significant additional conjugation and so lacks any substantially increased thermodynamic drive towards its formation.

The Grignard reagent is quite capable of inducing this β-elimination and on statistical grounds appears more favourable than the addition to the ketone as there are four times the number of α-hydrogens as ketone sites. It is even feasible that both occurred. See fig. 43.

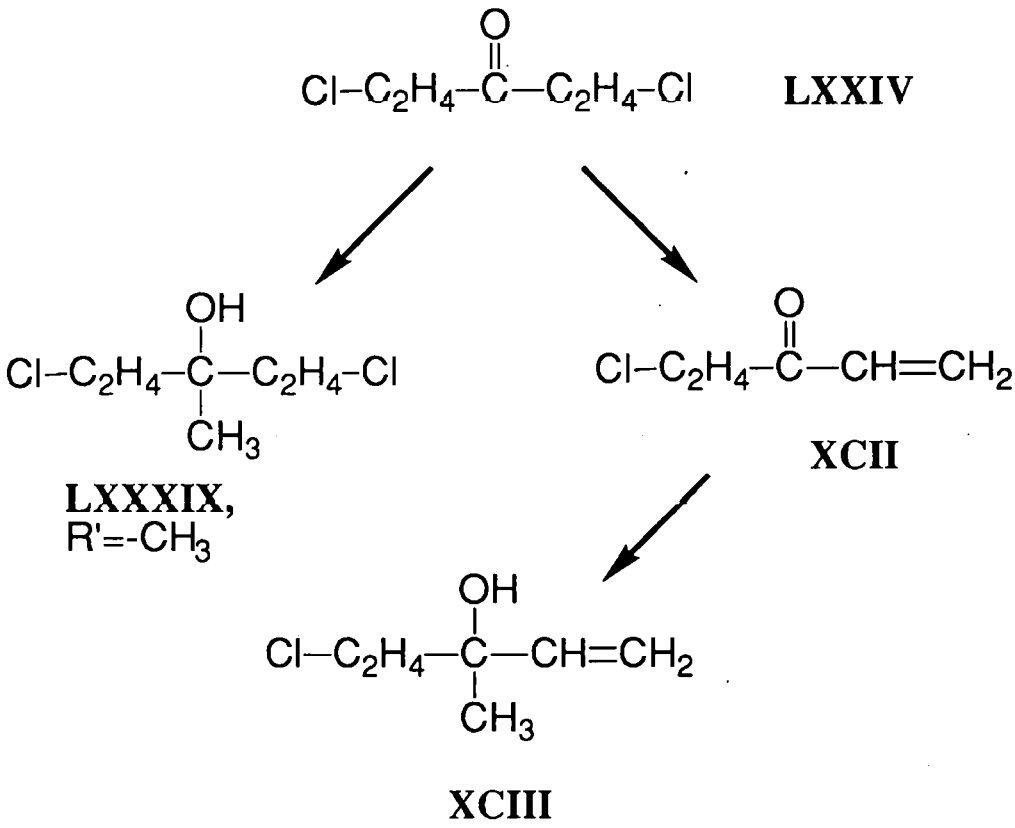


Fig. 43

Addition of the Grignard to the ketone site of the α,β-unsaturated ketone, XCII, is only one possibility, as addition to the β-carbon of the alkene site is also favourable with the oxygen being able to stabilise the subsequent α-carbon anion formation with the enolate (CXIII). See fig. 44.

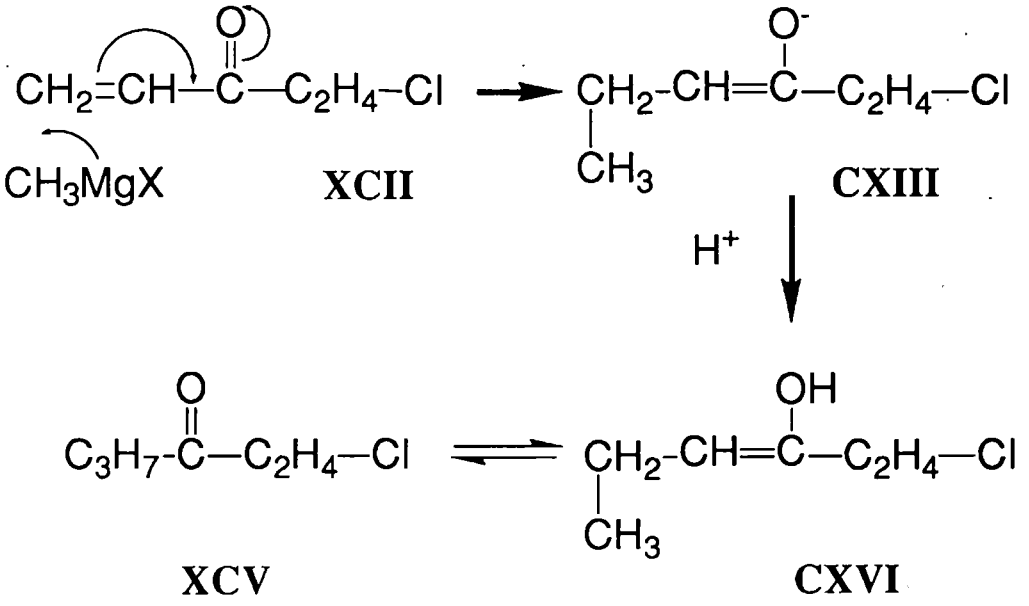


Fig. 44

The 1-chlorohexan-3-one (XCV) or its enol (CXVI), will, of course, then be able to undergo either direct addition with the Grignard or β-elimination with or without subsequent addition of a Grignard reagent which gives the possibility for further impurities.

However, the main side-product, as already mentioned, was a plastic, inert, sponge like material. This suggested that there had been some degree of polymerisation. As this material had been formed before quenching of the Grignard, the Grignard reagent(s) themselves would be expected to be the main cause of the polymer. There are many possible pathways to the polymerised side-products and without analysis of the product, these will only remain speculative.

Enolisation of 1,5-dichloropentan-3-one (LXXIV) would be expected to be slow but once formed would generate a nucleophilic initiator towards polymerisation with the α,β-unsaturated ketone which is activated towards nucleophilic attack by the electron withdrawing carbonyl and the remaining 2-

chloroethyl group. The Grignard reagent could, of course, accelerate the formation of the enolate by removal of one of the α-hydrogens.

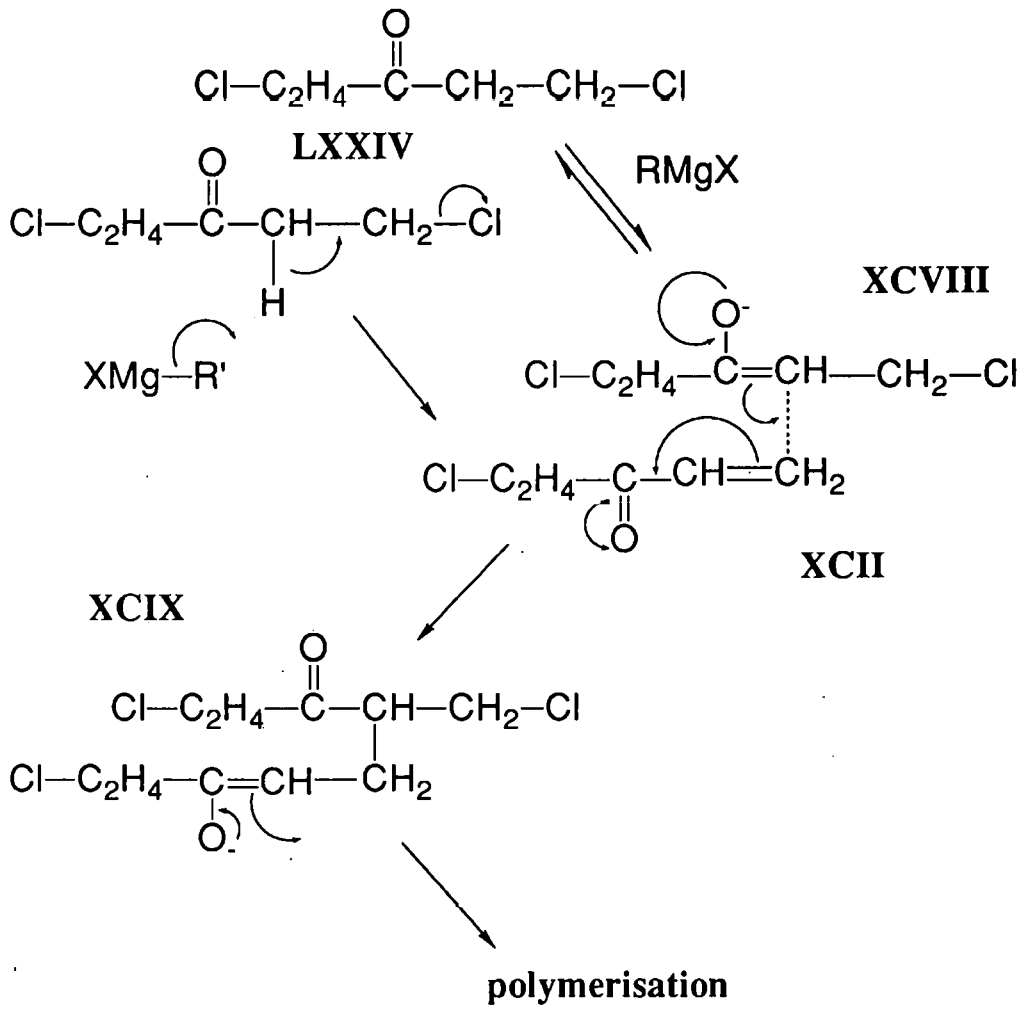


Fig. 45

After formation of the dimer, XCIX, the enolate anion would be able to promote further addition and, hence, polymerisation (steric hindrance permitting). See fig. 45.

Alternatively, the α,β unsaturated ketone, XCII, may be first alkylated by the Grignard reagent giving an activated nucleophile, XCVI, which would then be able to initiate polymerisation in the same manner as the above example only this time, the dimer CXVII would be formed. See fig. 46.

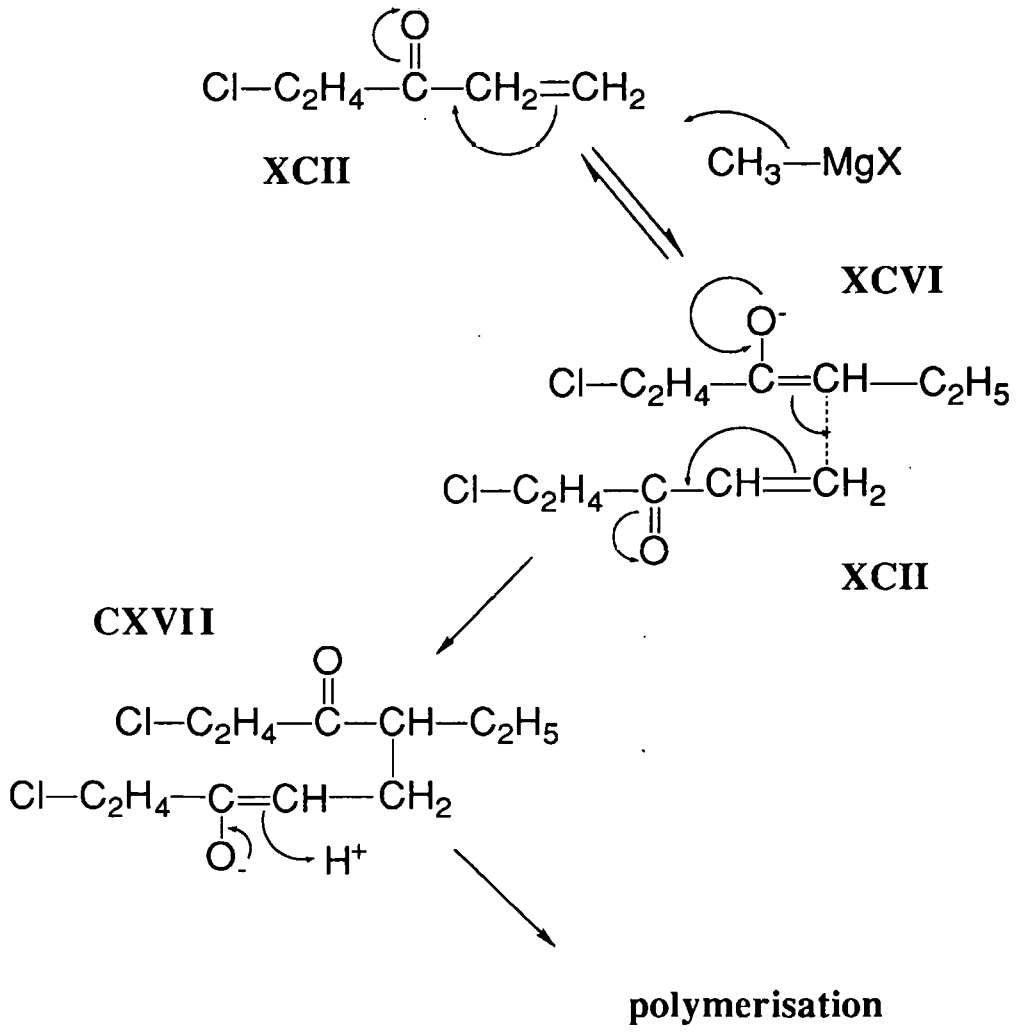


Fig. 46

A third source of impurities is solvolysis of the primary chlorine of the desired alcohol XXXIX, R' = -CH_3, during the quenching of the Grignard. See fig. 47. (This of course, could lead to either the diol (XCVII) or, following further solvolysis of XCVII, the triol).

Solvolysis of the primary chlorine would be expected to be slow but evidence for the mono-substituted alcohol (XCVII) exists. High boiling fractions of the

distilled reaction products contained a particularly high proportion of the primary alcohol as seen by ^1H NMR spectroscopy.

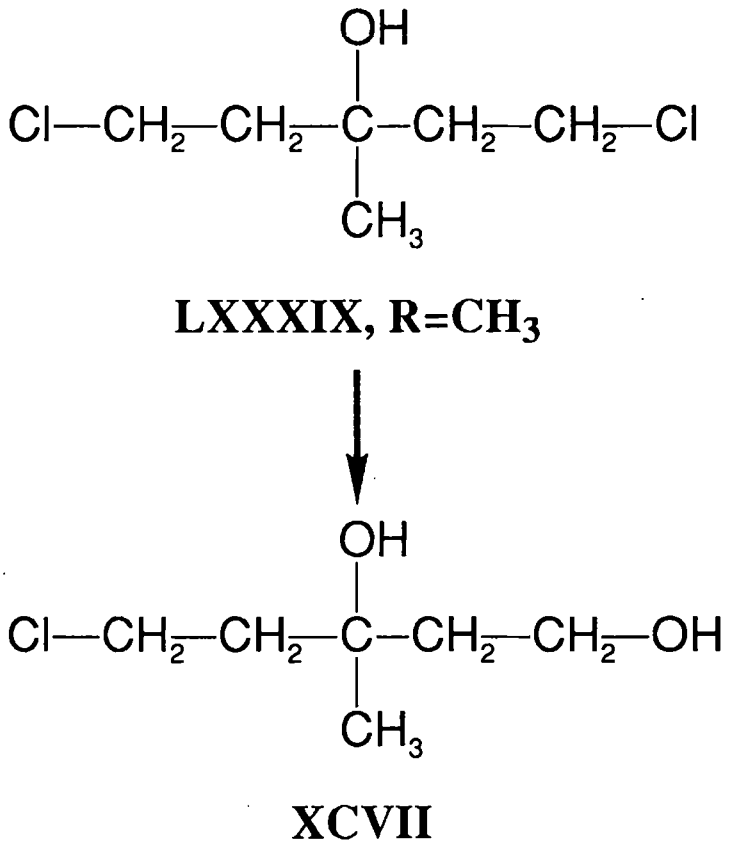


Fig. 47

Evidence for the formation of these by-products (XCII, XCIII, XCVII, XCIX, CXVI & CXVII) is also seen in the ^1H NMR.

In the examination of the impure reaction mixtures the only alkene protons seen in the ^1H NMR were found between δ5.00 and δ6.20 implying that there was no conjugation: the α,β-unsaturated ketone system giving proton resonances between δ6.90 and δ7.80 as observed in the by-products of the subsequent formation of the trihalide (see section II.2.1.3). This suggests that the excess Grignard reagent which was used, utilised all the ketone available, including XCII, aided by refluxing.

A methyl signal 2Hz downfield from that seen with LXXXIX, R' = methyl was attributed to the methyl of XCIII, and a signal due to alkene protons of XCIII was seen between δ5.00 and δ6.20.

Evidence for polymerisation is also seen in the ^1H NMR spectrum: the ratio of methylene protons with an adjacent chlorine atom to methylene protons without, is lower in reaction mixtures which have been refluxed. Theoretically, the ratio of the β-methylene protons to the α-methylene protons should be equal in the monomer but reduced in the polymers. The exact value will obviously depend on which, and how many, repeating units comprise the polymer but the value actually found was 0.75. The dimer was never isolated from the other by-product contaminants of LXXXIX, R' = methyl, and so its exact identification is not known.

Evidence for the existence of the primary alcohol, XCVII, is seen in the ^1H NMR spectrum of the mixture of LXXXIX, R' = methyl and XCVII. A triplet upfield of the chloromethyl protons at δ3.25 is seen together with a sharp singlet at δ3.45 which disappears with a D_2O shake (in addition to the tertiary alcohol peak at δ1.90) and a tertiary methyl signal 2Hz upfield from that seen in LXXXIX, R' = methyl.

Thus it is possible that, at least, the unsaturated alcohol, the polymer and the primary alcohol were made as side-products.

II.2.1.3 Synthesis of the Halo-Carbon Mustards

The alcohol LXXXIX was halogenated to give the trihalide (XC) using either concentrated hydrochloric acid and zinc chloride or with hydrogen bromide in glacial acetic acid, to give the bromide (XCI). The crude reaction product was unstable and had to be purified by medium pressure fine silica chromatography.

Bromination of LXXXIX was expected to give a more reactive mustard (XCI) than the corresponding chlorinated compound (XC) because of the weaker nature of the carbon-bromine bond. However, the greater ease of dissociation is more likely to lead to alkene impurities (although rearrangement products would not be expected). Indeed, XCI was more prone to spontaneous decomposition and was thus harder to make and keep pure. (Bromination was also more difficult to effect requiring the stronger acid).

The product was again identified by the shift in α-methylene protons this time downfield to: δ2.30 (4H,m) for XCI, R' = methyl; and to δ2.25 (t, $^3J_{H,H}$ = 6Hz) for XC, R' = methyl. The α-methylene proton triplet of the bromine compound, XCI, was seen to resolve into a seven peak multiplet which probably represents second order splitting. This is believed to be due to the diastereotopic relationship between the two protons making up the α-methylene signal becoming apparent in the bromine halide.

The substituted forms of XCI made include the methyl, ethyl and phenyl (X = chlorine only) derivatives. The 3-bromo-1,5-dichloro-3-methylpentane was used for the majority of the subsequent reactions, with the intention of returning to the possible analogues (at the 3-position) after successful elucidation of the complete phosphoryl carbon mustard synthesis.

It is interesting that the impurity in the alcohol which gave rise to the triplet at δ3.25, which was thought to be a primary alcohol, C, was also chlorinated giving XC when included in the reaction mixture; the methyl signal of XCVII moved downfield to δ1.55 in the ^{1}H NMR. See fig. 48.

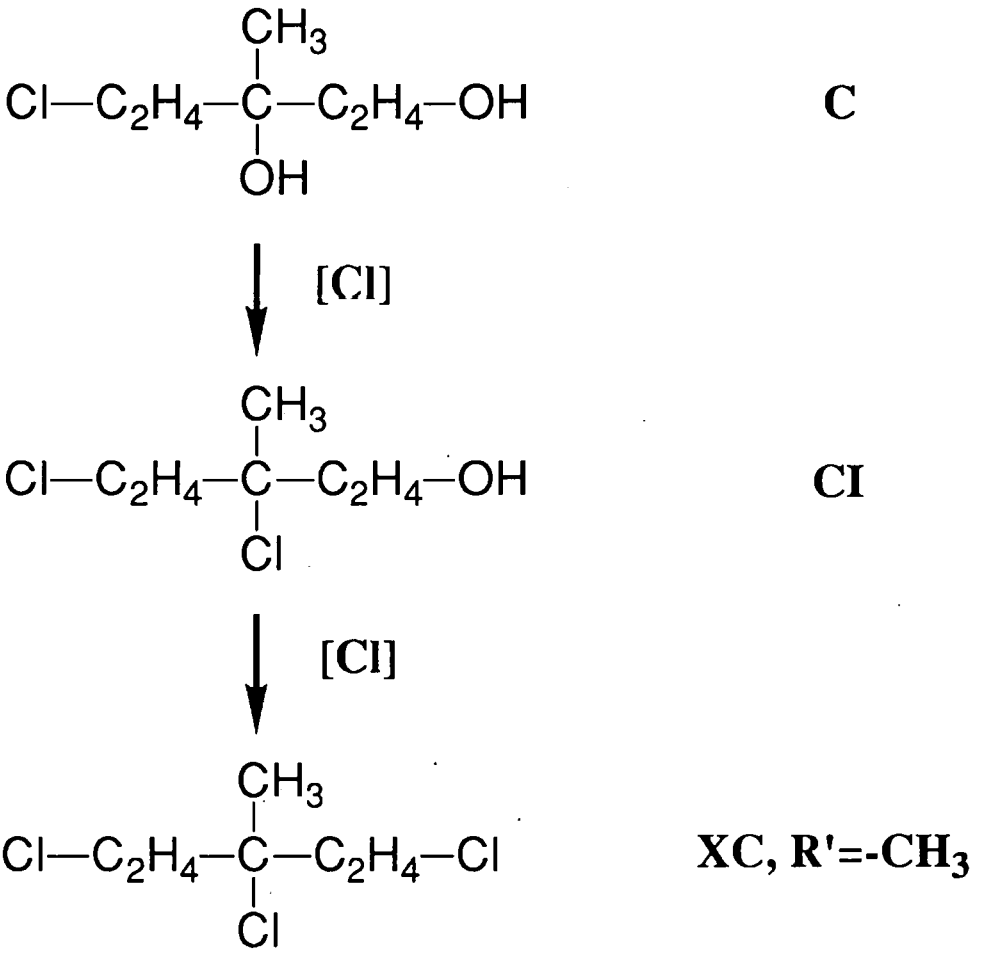


Fig. 48

II.2.1.4 Other Methods of Synthesis of Carbon Mustard Halides

Other synthetic routes towards the carbon mustard halide were tried. One such route involved the tetrahydropyran protected 2-bromoethanol magnesium Grignard reagent (XCIV) and the appropriate ester. See fig. 49. The

protected alcohol 2-(2-bromoethoxy)pyran was made from dihydropyran and 2-bromoethanol. Reaction of the protected bromo-alcohols (CII) with magnesium was not forthcoming, however, and some inhibitory, non-bonding bromine-pyranyl oxygen interaction was tentatively suspected.

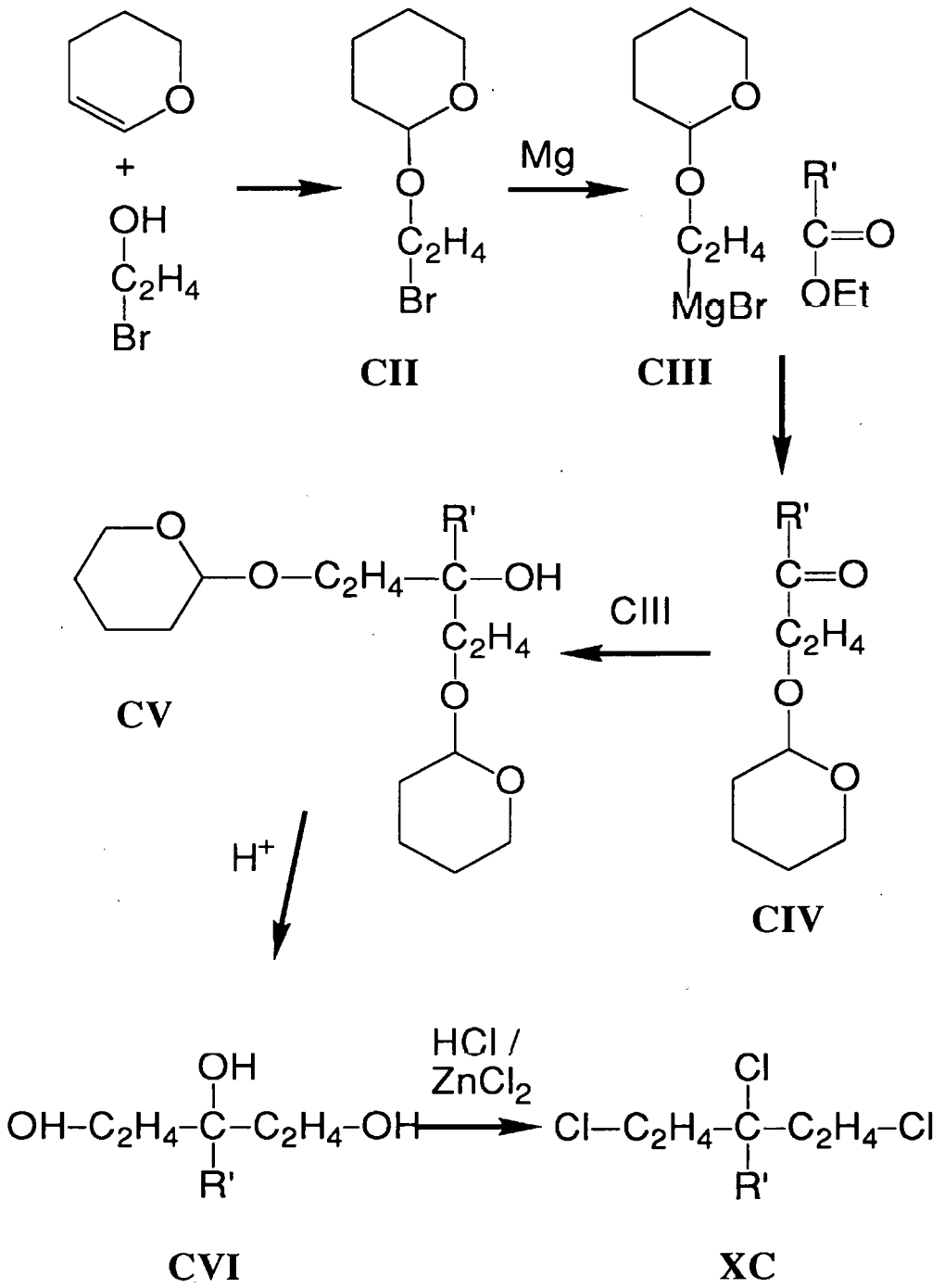
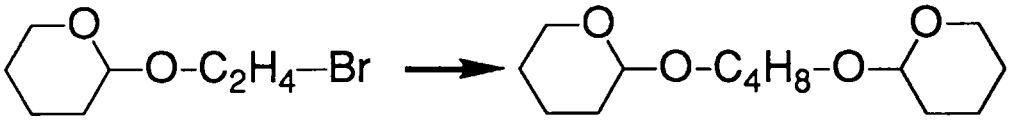


Fig. 49

Projected synthesis of tri-halide mustard using tetrahydropyran protected alcohols.



CII **CVII**

Fig. 50

Wurtz coupling of the protected 2-bromo-ethanol in the presence of n-BuLi.

Reaction with *n*-butyllithium was tried as an alternative to the formation of the Grignard but resulted in the Wurtz coupled product, 1,4-bis(tetrahydropyran-2-yloxy)butane (CVII), in stoichiometric amounts. See fig. 50.

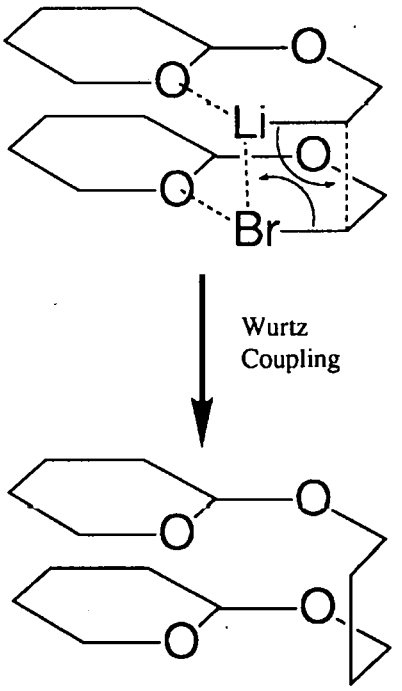


Fig. 51

One possible explanation for this is an intramolecular lithium-pyranyl interaction, which is able to effect a displacement reaction, normally only seen with the more electropositive organosodium compounds. A favourable reaction conformation may be attained by an intermolecular interaction thus lowering the activation energy for the formation of the Wurtz coupled product. See fig. 51.

Dihydropyran was used to protect the alcohol groups as the resulting ether is stable towards alkali but can be readily converted back to the alcohol with dilute acid. It is possible that the tetrahydropyran ethers were responsible for the difficulties of these reactions, as mentioned above, and so attempts were made with an alternative protecting group, trimethylsilyl. Since the trihalides became available via the Friedel Crafts route this approach was abandoned. See section II.4.

II.2.2 Addition of Phosphoryl Group as a Nucleophile

As discussed in section I.5.3.3, trivalent phosphorus compounds, by virtue of their lone pair of electrons, can act as nucleophiles. The Arbuzov reaction makes use of the nucleophilicity in the formation of phosphorus-carbon bonds, usually by the reaction of phosphites with organic halides. However, the Arbuzov reaction proceeds via an S_N2 mechanism[63] and the unmodified reaction was not expected to lead to condensation of the carbon mustard halide (LXXXVIII) to a phosphoryl group because of the steric hindrance offered by approach to the tertiary carbon atom.

In agreement with this, trialkyl phosphites would not react with the carbon mustard halides despite lengthy refluxing in aprotic solvents and later even without solvents at all. The bromine carbon mustard was used because of the

better leaving group properties expected of a bromide over a chloride due to the bromine's poorer basicity.

It was considered that, by improving the leaving group qualities of the substituent on the tertiary carbon, it might be possible to promote carbon-phosphorus bond formation. It was postulated that the reaction of the carbon mustard halide with a poorly nucleophilic silver salt, such as perchlorate, tosylate or triflate,[71] might have the effect of activating the mustard, as these are very good leaving groups. Thus attempts were made to carry out the Arbusov reaction with the carbon mustard attached to one of these leaving groups. See fig 52.

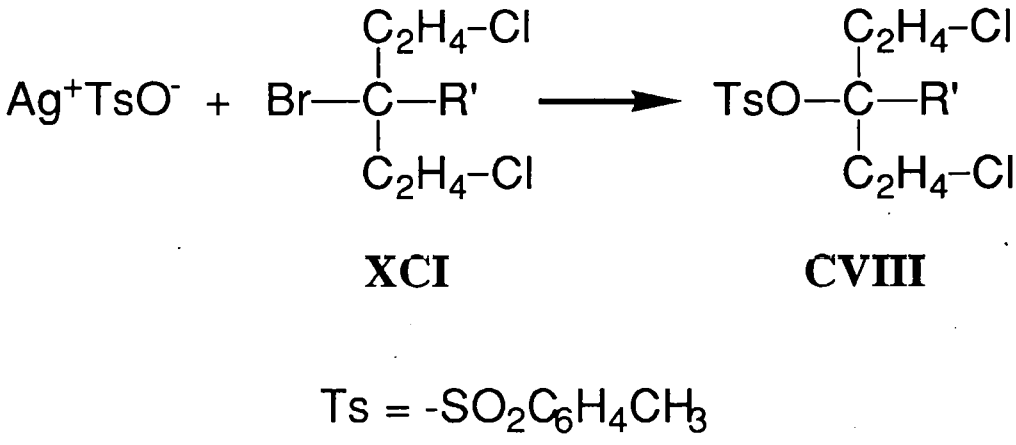
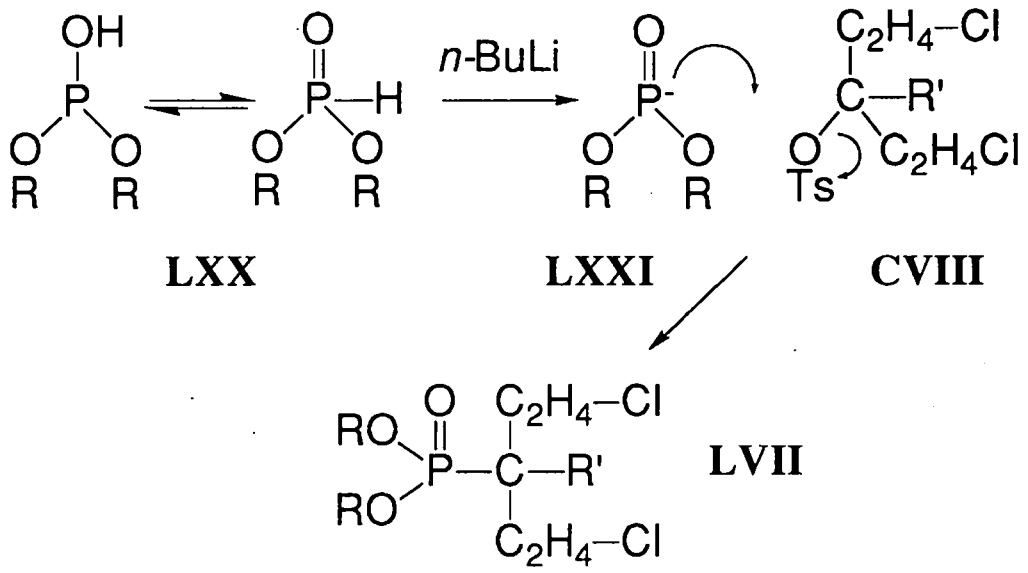


Fig. 52

In the event, there was no subsequent reaction of CVIII with the trialkyl phosphite under these conditions.

In case the mustard was still not activated enough, reaction of the suspected mustard tosylate was tried with a pre-formed dialkyl phosphite anion. See fig. 53. But again, no evidence for reaction was obtained: despite the tosylate's good leaving group properties, the steric hindrance offered to an approaching

nucleophile may be too great: the tosylate adding to the already bulky mustard as demonstrated by the failure of the Arbuzov reaction. The bromide ion itself is a good leaving group and it was just possible that phosphite anion attack on the carbon mustard halide would bring about bromide loss. The chloride ion offers less steric hindrance to an approaching nucleophile but against this, the carbon-chlorine bond is stronger and a chloride ion is a less good leaving group than a bromide ion. However, as discussed later (section II.2.3) the phosphite anion appeared to cause β-elimination rather than substitution.



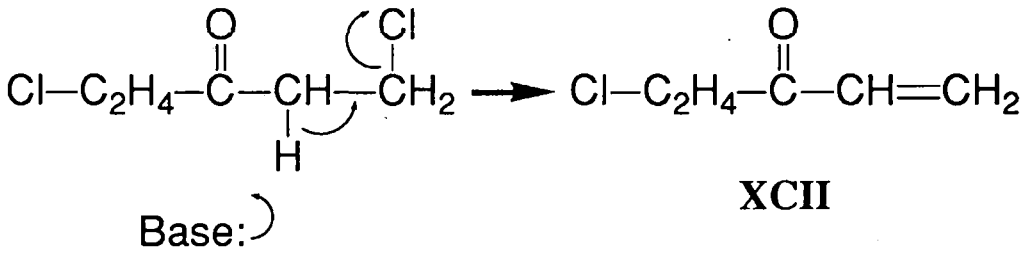
Memory Low!

Fig. 53

II.2.3 Hydroxyl Carbon Mustard Phosphonates

Concurrent with the above approach, attempts were made to make 3-hydroxyl phosphoryl carbon mustards, LXXVI (see fig. 32). It was thought that phosphoryl anion attack on the 1,5-dichloropentan-3-one (LXXIV) would be less susceptible to effects of steric hindrance than attack on the halide carbon

mustards, LXXXVIII, as the site of attack on the quarternary carbon atom is sp^2 hybridised instead of sp^3 hybridisation in LXXXVIII. Initial attempts with this reaction, involving the use of *n*-butyllithium (and later, potassium fluoride) to abstract a proton from a dialkyl phosphite, merely promoted β-elimination of hydrogen chloride from LXXIV and the formation of unwanted unsaturated products. β-Elimination was either caused by excess *n*-butyllithium or the phosphite anion itself giving 5-chloropent-2-en-3-one (XCII). See fig. 54.



XCII

Base: = KF:, $^-PO(OR)_2$, or *n*-BuLi

Fig. 54

With the phosphite used in excess the ketone should then only be exposed to the phosphite anion, and not to the *n*-butyllithium base. However, when this was tried, the reaction products were of a similar nature suggesting that the phosphite anion was indeed able to cause β-elimination of the ketone by itself.

A further problem was encountered when a preliminary reaction with the model ketone, pentan-3-one, yielded a product that was heavily contaminated by saturated hydrocarbons (broad peak at δ1.6 and δ1.0 in the ^1H NMR).

These saturated hydrocarbons could not be separated despite prolonged periods of time under vacuum and attempted purification with fine silica column chromatography. (Gas liquid chromotography merely revealed an unresolved mixture of late compounds).

Similar results were also obtained with 4-chlorobutan-2-one, which was synthesised from acetyl chloride by Friedel-Crafts acylation of ethylene. Significant quantities of saturated hydrocarbon contaminations, together with extensive decomposition products (none of which could be separated), prevented definitive identification.

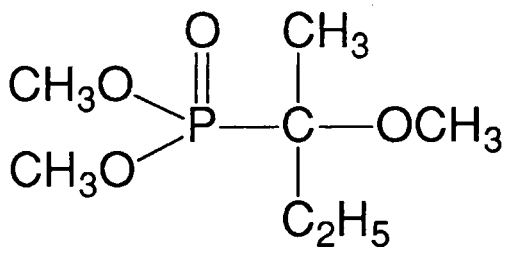
These series of reactions which produced substantial and irremovable quantities of contaminant aliphatic peaks in the NMR tends to suggest that the butyl element of the base had condensed with the formed phosphonate. As discussed above, and shown in figs. 42, 45, 46 & 54, the formation of the α,β-unsaturated ketone, XCII, could be easily brought about by bases such as butyllithium. This product is then susceptible to attack by either the phosphonate again or *n*-butyllithium itself, which in the case of the α,β-unsaturated ketones, would be expected to give the Michael addition product.

In an attempt to obtain a less complex reaction mixture, a series of reactions was done using *t*-butyllithium. Analysis of the products showed that *t*-butyl peaks were in evidence at various chemical shifts in the ^{1}H NMR, suggesting that the base was indeed being incorporated into the reaction products despite *t*-butyllithium's poor nucleophilicity.

Foucaud has reported an elegant method for condensing dialkyl phosphites with ketones using either potassium or caesium fluoride to catalytically abstract a proton from the phosphite.[65] When this work was repeated, it was found to work well for the ketones which weren't too sterically hindered. The

reaction with acetone and dimethyl phosphite gave dimethyl 2-(2-hydroxypropyl)phosphonate in 97% yield, while with pentan-3-one and dimethyl phosphite, a long period of reflux was required to give only a poor yield of 32%. Thus steric hindrance could be expected to be a problem with 1,5-dichloropentan-3-one when used in the Foucaud method.

An interesting result from the use of potassium fluoride catalysed phosphite anion addition was found with butanone. One of the products isolated was dimethyl 2-(2-methoxybutyl)phosphonate (LXXVII, R'= methyl). See fig 55.



LXXVII, R'=-CH_3

Fig. 55

The excess dimethyl phosphite had apparently acted as a methylating agent. However, with diethyl phosphite the expected product, diethyl 2-(2-hydroxybutyl)phosphonate, was obtained. It is unclear why some systems produce an alcohol and others the ether but presumably the size and reactivity, of the phosphite alkyl group is of some importance.

Despite the results it was hoped that this method might be acceptable for 1,5-dichloropentan-3-one and so lead on to the hydroxyl carbon mustard phosphonates. However, it soon became apparent that potassium fluoride was also a strong enough base to induce β-elimination of the 2-chloroethyl

moieties, giving the α,β-unsaturated ketone which is open to further condensation with the phosphite.

Further investigation showed that the ketone (LXXIV) alone, after only a few minutes exposure to potassium fluoride, decomposed completely in a noticeably exothermic reaction into many unidentifiable products. Two products that can be expected are explained by the ketone undergoing β-elimination to form the α,β-unsaturated ketones, 5-chloropent-1-en-3-one (XCII) and penta-1,4-dien-3-one (XCIV): evidence for these two compounds is seen in the ^{1}H NMR with conjugated alkene proton resonances between δ6.90 and δ7.80.

Further reaction of these alkenes to form dimers and trimers, catalysed by the potassium fluoride, could be expected, leading to the many reaction products in a similar manner to the Grignard reactions of 1,5-dichloropentan-3-one (See figs. 42, 45 & 46). Evidence for conjugation is also seen with the carbonyl infrared absorption of 1,5-dichloropentan-3-one. The carbonyl peak at 1720cm^{-1} due to the unsaturated ketone becomes broad with many shoulders and there is a substantial increase of an absorption at 1620cm^{-1} which appears in the fresh 1,5-dichloropentan-3-one as an ubiquitous impurity. This may be due to a bis-conjugated ketone system of either the monomer elimination product or of a small polymer although it is a little low (expected range 1685-1665cm^{-1})[69] (Interestingly, an α,β-diketone would have been expected to have an absorption in the range 1640 cm^{-1} - 1535 cm^{-1} but it is difficult to see how such a compound could be formed without the loss of a 2-chloroethyl group - which is unlikely).

As a result of these experiments, it became clear that the route using potassium and caesium fluoride was found to be unsuitable for 2-chloroethyl ketone compounds.

II.2.4 Mono-Functional Phosphoryl Carbon Mustards

Mono-functional phosphoryl carbon mustards - that is, compounds containing just one 2-chloroethyl moiety - might not be expected to show significant biological activity (see section 1.2.2) but the availability of such compounds would at least be useful as kinetic references to the bi-functional carbon mustards. Moreover, 2-chloroethyl ketones have already proved their ability to act as alkylating agents via the α,β-unsaturated ketone which may indeed allow compounds of the class LXXX (fig. 33) to have biological activity.

The compounds within this family of mono-functional agents have the general form LXXXI (see fig. 33) and might be synthesised from two routes also shown in fig. 33. Synthesis via the ketone LXXX from the acylchloride would be apparently devoid of significant steric hindrance which plagued so many of the parent compounds: a substituent being introduced by a Grignard reagent's attack at the carbonyl. In addition, this route allows study of the phosphoryl, carbonyl mono-functional carbon mustard, LXXX.

In the event phosphite anion attack on the acylchloride in the first stage yielded ethyl 3-chloropropionate with no evidence for the desired ketone, LXXX. A proposed scheme for this reaction is shown in fig. 56.

Upon extraction of the reaction mixture using methylene chloride, the infrared spectrum of the product showed a variable, broad absorption between 3400-2800cm^{-1} and a slightly broad, rough peak at 1735cm^{-1} - characteristic of a carboxylic acid.[72] These absorptions disappeared when the products had been washed with sodium bicarbonate solution.

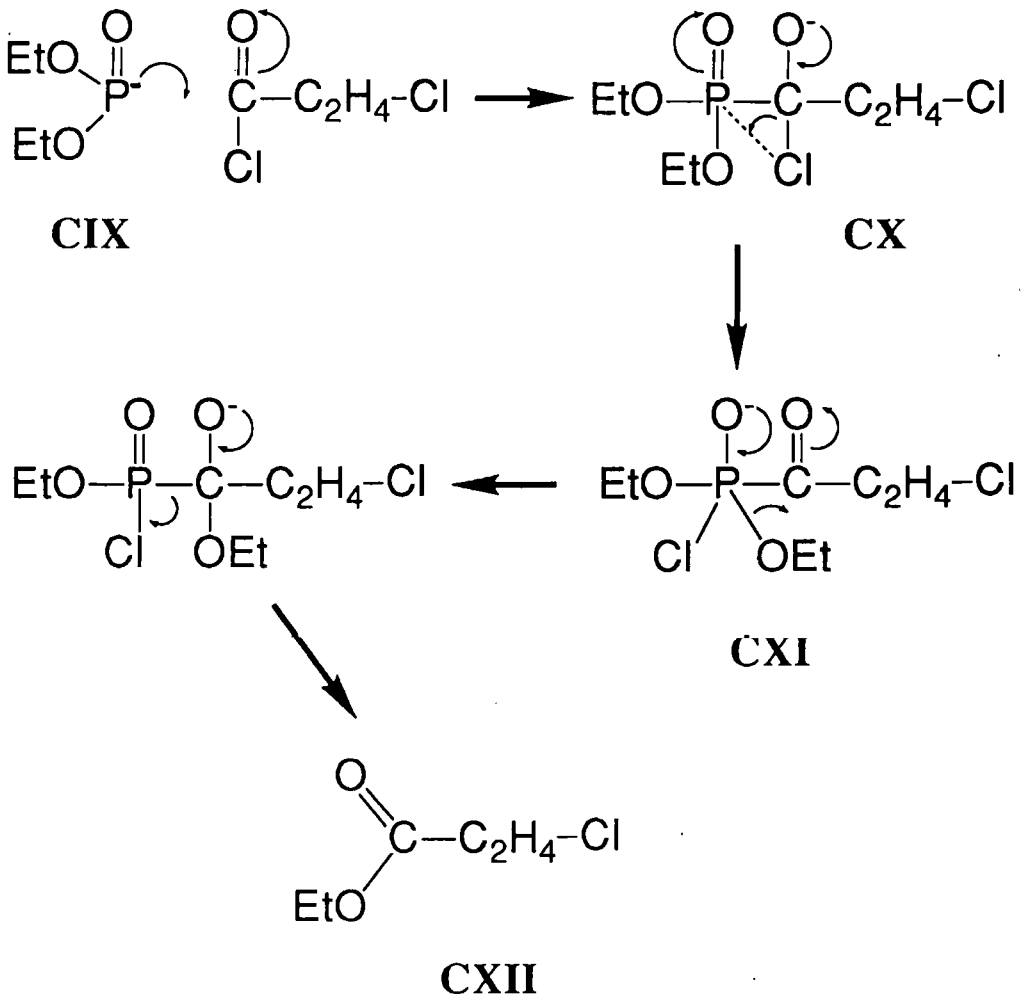


Fig. 56

Another weaker carbonyl absorption at 1825cm^{-1} was also seen and was suspected of being the anhydride of the above carboxylic acid. The formation of two compounds was supported by the intensities of the corresponding peaks in the ^{1}H NMR. The two triplets at δ3.75 and δ2.75 assigned to the protons of the two methylene groups in the 2-chloroethyl group had a ratio of 1.0 under all conditions. The quartet at δ4.10 and the triplet at δ1.30, which were associated with ethoxy groups of the phosphoryl group, also remained in a constant ratio of 1.0. However, these two pairs of peaks did not remain in constant ratio to each other but depended on the conditions of purification (range 1.1:1.7) suggesting the product to be a mixture of ethoxy and 2-chloroethyl containing compounds.

However, fine silica column chromatography, with a variety of solvent systems, failed to separate the phosphorus components completely from the ethyl 3-chloropropionate but the varying proportions of the peaks in the NMR continued to support the idea that these were mixtures of compounds rather than varying types of compounds.

The conclusion from this series of reactions is that the diethyl phosphite donates an ethoxy group to the acid chloride to give the propionyl ester. During purification, the washing of the unreacted acid chloride - which appears to be less susceptible to hydrolysis than the corresponding non-chlorinated acid chlorides - is partially hydrolysed to the acid and this reacts with the remaining acid chloride to give the anhydride. These then decompose on the work up into a variety of compounds as well as many other phosphorus by-products.

II.2.5 Addition of Phosphoryl Group as an Electrophile

The susceptibility of pentavalent phosphorus to nucleophilic attack can be enhanced by including a good anionic leaving group within the molecule. For bonding of the carbon mustard moiety to this, the quaternary carbon must be induced to become electron rich.

A common method for producing an electron dense carbon is by way of an organometallic compound, or more specifically, a magnesium Grignard reagent. Formation of the Grignard reagent from the already available carbon mustard halide XCl, would theoretically permit addition of the phosphoryl moiety via diethyl chlorophosphonate (LXIV). See Fig. 57.

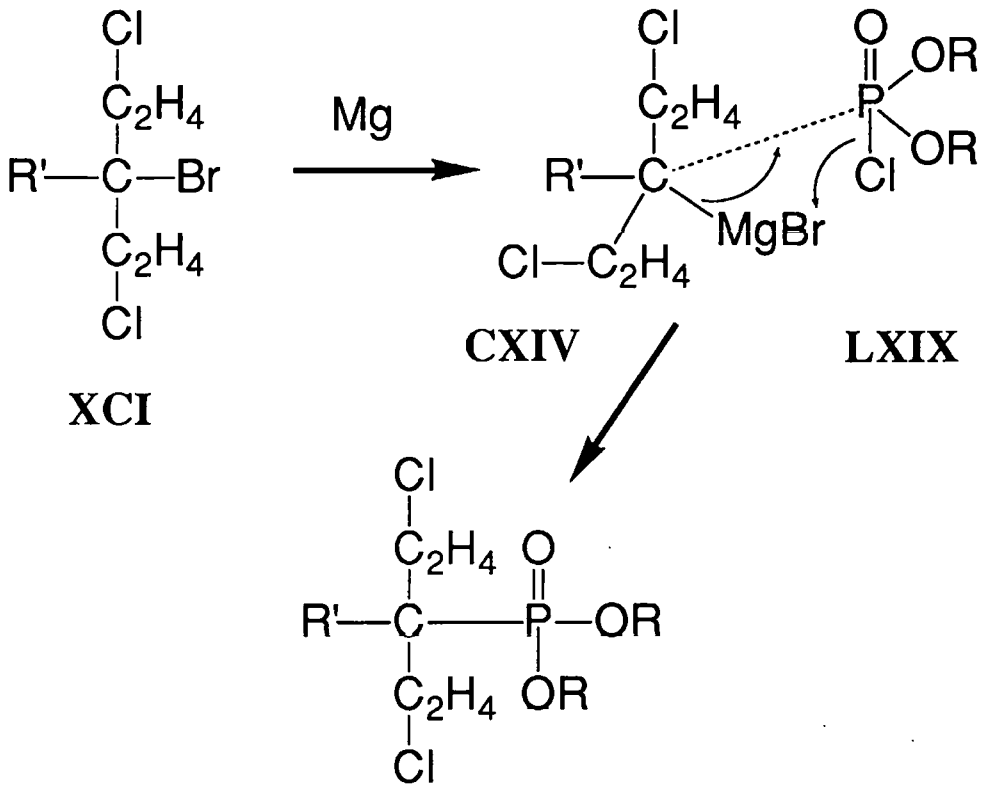
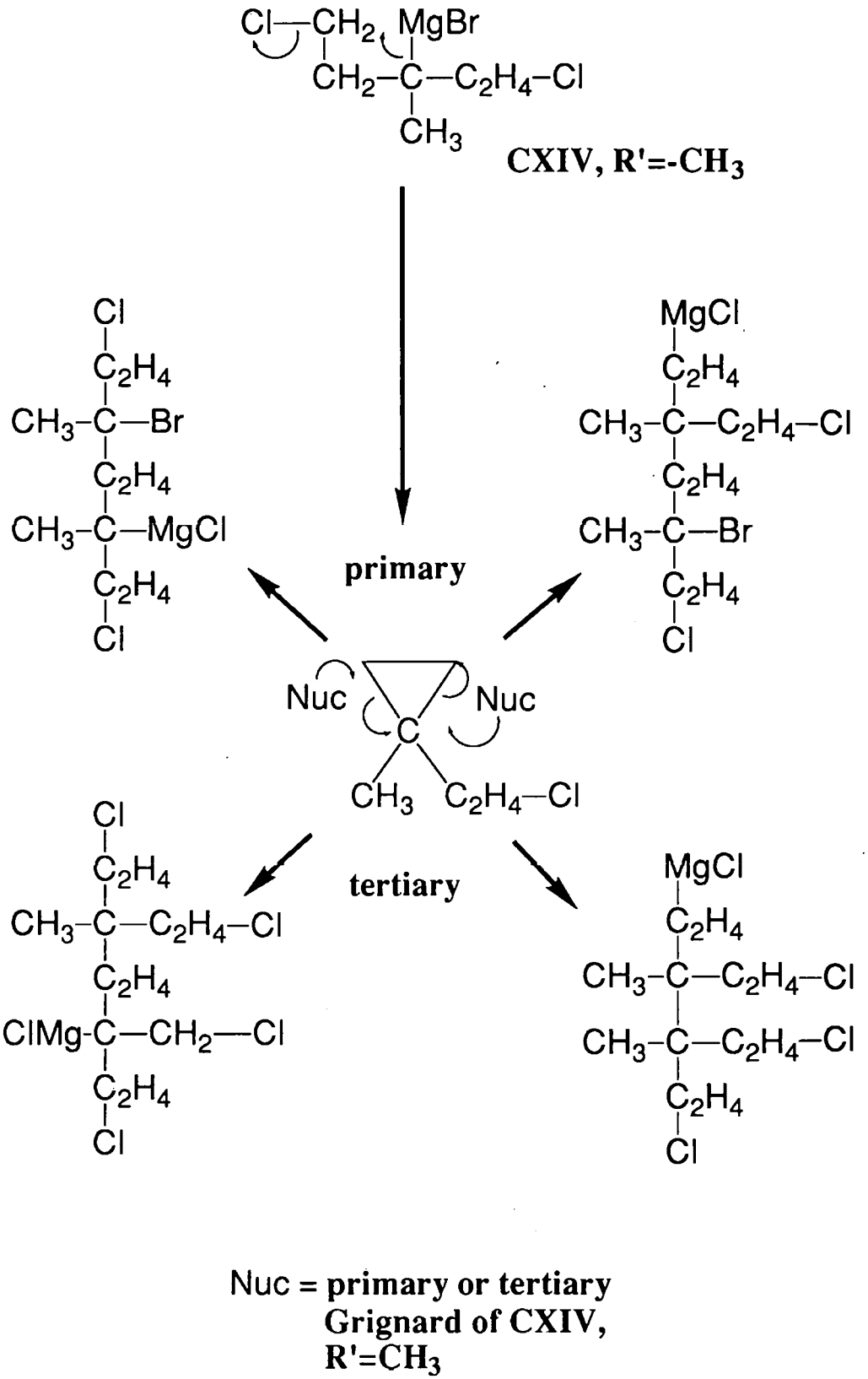


Fig. 57

However, for the scheme to work, the Grignard reagent, CXIV, must be made specifically at the quarternary carbon atom rather than at the primary centres. As discussed earlier, the carbon-bromine bond is more highly polarisable than the carbon-chlorine bond and so should be more susceptible towards attack by the magnesium (see section II.1.2.3). In favour of primary site specificity is the fact that the tertiary site is a lot more sterically hindered even for the approach of the small magnesium.



Nuc = **primary or tertiary Grignard of CXIV, R'=CH_3**

Fig. 58

Additionally, the development of the electron rich centre at C_3 is dependent on the resulting inductive effects of the 2-chloroethyl and substituent group, R'.

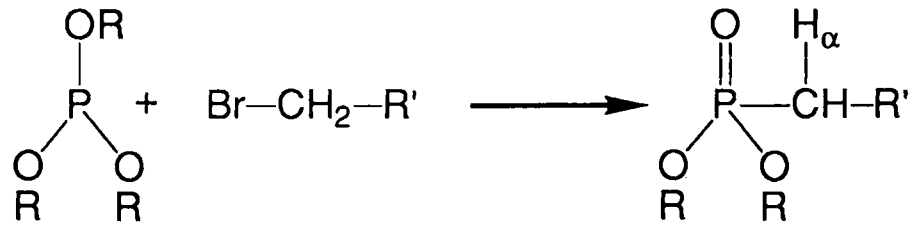
When this strategy was attempted, the carbon mustard bromide (XCI) did appear to react with the Grignard after gentle warming, but addition of the chlorophosphonate produced a heterogeneous mixture of reaction products which were not readily identifiable. However, the same mixture was obtained apparently independent of the added electrophile: dimethyl or diethyl chlorophosphonate, acetone or even water.

It was thus evident that the magnesium was inducing a decomposition of the carbon mustard bromide either inter- or intra-molecularly. It is possible that as the Grignard reagent induces an electron dense tertiary carbon, by design, this may be causing an anchimeric loss of the primary chlorine (*cf.* nitrogen mustards). This may result in the formation of a cyclopropyl derivative which would then be open to nucleophilic attack from either the primary chloride or tertiary bromide Grignard reagents.

In addition, the favourably unhindered site of attack at the methylene carbons is reduced by the subsequent formation of a tertiary carbanion. Hence the quarternary carbon might also be a site of attack, despite the greater steric hindrance, by either of the two Grignard nucleophiles. See Fig. 58.

II.3 ADDITION OF 2-CHLOROETHYL GROUPS TO A PHOSPHONATE SKELETON

There is a wide range of simple phosphonates which are either available commercially or can be made simply by the Arbuzov reaction.[63] See figs. 29 & 59.



LXVII **CXV** **LXI**

Fig. 59

There are certain difficulties with the Arbuzov reaction, however. The first of these is the inability for triphenylphosphite to undergo the Arbuzov reaction at all, the phenyl group in the intermediate cation, being unable to undergo nucleophilic displacement. Secondly, the alkyl halide by-product from the reaction might be more reactive than the reagent halide. This was indeed found with the attempted synthesis of dimethyl ethylphosphonate using trimethyl phosphite and ethyl iodide. The displaced methyl iodide was simply more reactive than the ethyl iodide and dimethyl methanephosphonate was exclusively produced. However, the Arbuzov reaction is still a very useful and versatile route of synthesis.

For the same reasons that α-hydrogens to a carbonyl group are acidic (*cf*: carboxylic esters), α-hydrogens to a phosphoryl group can also be expected to have a relatively acidic pK_a: LXII, fig. 50, for example. Warren[64,73-4] has already extensively made use of the acidity of the α-hydrogens with the preparation of the diphenyl phosphine oxides: *n*-butyllithium being used to abstract a proton to form the resonance stabilised anion LXII, Fig. 26. The phosphonate anion LXII would then subsequently be expected to attack an electrophile which would allow the addition of the two 2-chloroethyl groups to yield the carbon mustard phosphonate LVII seen in Fig. 26.

This synthesis is attractive for three main reasons:

(i) the strategy is relatively short and simple;

(ii) it is free from all the problems encountered through activation of the quaternary carbon with the late conjugation of the phosphoryl group; and

(iii) the relative ease of introducing the various substituents, R'.

Against these advantages, it must be remembered as discussed in section II.2, the reactions and purifications of phosphoryl containing compounds can be difficult especially if the reaction is not clean.

II.3.1 Direct Addition of the 2-Chloroethyl Groups

It was hoped that, by a suitable modification to the 2-chloroethyl group to allow it to be susceptible towards electrophilic attack, that the mustard components could be conjugated directly to the pre-formed phosphonate anion, LXII. 2-Chloroethyl toluene-4-sulphonate might be expected to act as

a 2-chloroethyl donating group as the tosylate anion is well established as a good leaving group.

After the first addition of a 2-chloroethyl moiety, it was envisaged that a second equivalent of base would remove the second α-hydrogen from CXVIII and allow the formation of the bi-functional carbon mustard phosphonate. See fig. 60.

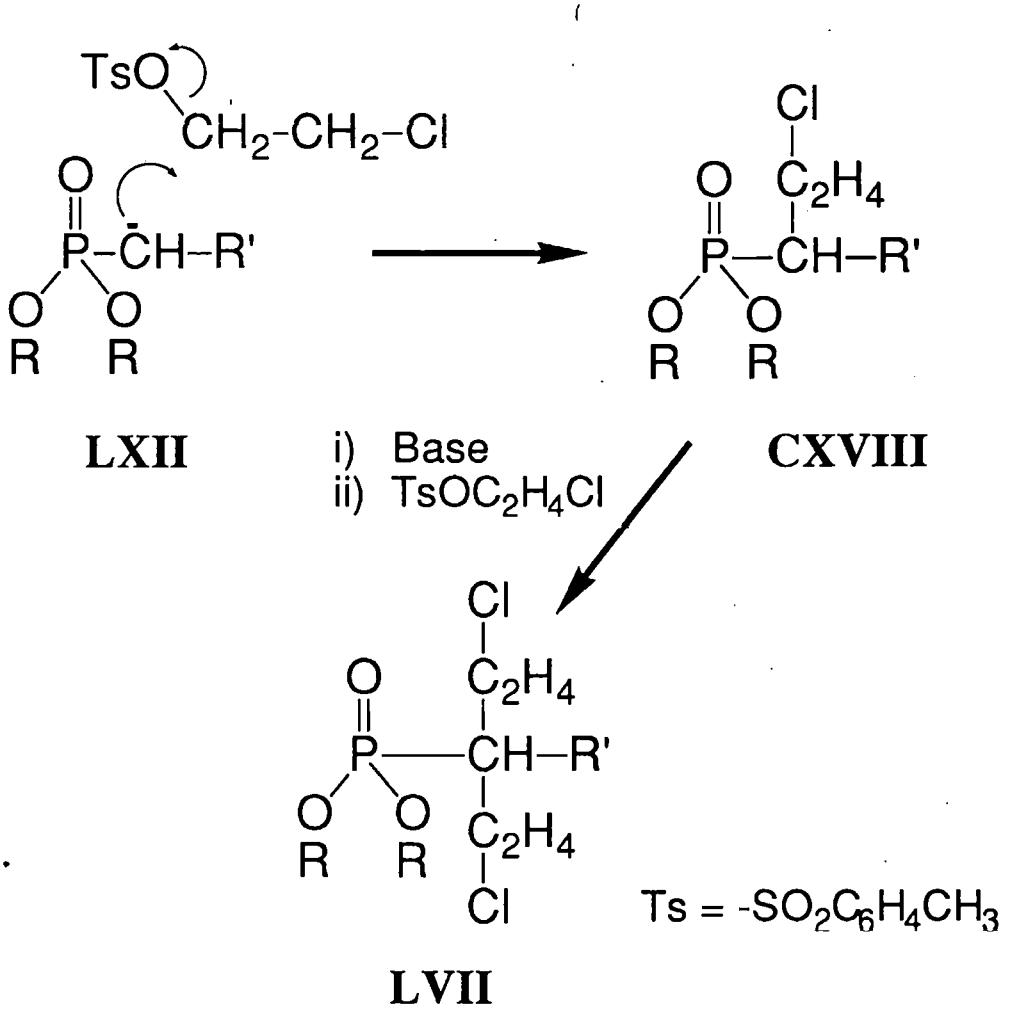
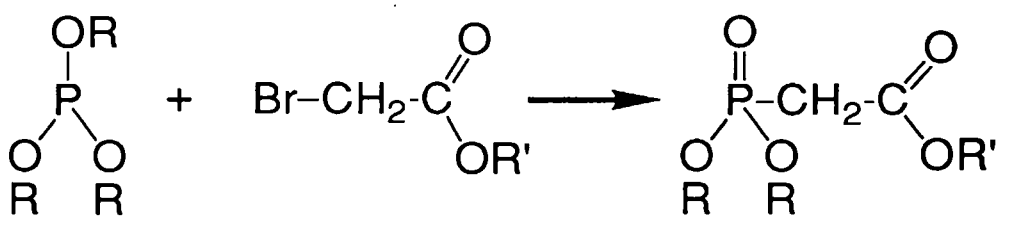


Fig. 60

The base *n*-butyllithium was chosen as the resulting lithium anion complex is soluble in tetrahydropyran, whereas sodium hydride was found to form a very

insoluble salt resulting in heterogeneous reaction mixtures. The addition of the base (*n*-butyllithium) to dimethyl benzylphosphonate produced a solution with a deep orange colour indicative of the formation of the anion but subsequent addition of the 2-chloroethyl tosylate failed to give the desired addition product. In fact, it appeared not to have reacted at all as identification of the products merely revealed many phosphoryl impurities and 2-chloroethyl tosylate. The formation of the anion was also tried in the presence of N,N,N',N'-tetramethylethylenediamine but this did not alter the results.



LXVIII **CXIX** **CXX**

Fig. 61

Arbuzov Condensation

Various trialkyl phosphonoacetate skeletons were also used in an attempt to synthesise the carbon mustard phosphonates by this route. Trialkyl phosphonoacetates are either available commercially or can be synthesised by the Arbuzov reaction with the trialkyl phosphite and the appropriate bromoacetate. See fig. 61.

The phosphonoacetate skeleton has two points in its favour for inclusion in a synthetic strategy towards the carbon mustard phosphonates:

(i) The α-hydrogens, analogous with those in malonates, are particularly acidic because of the neighbouring phosphoryl and carbonyl groups; and

(ii) the ester function, which would remain in the final mustard, would be a useful entry point for other members of the carbon mustard phosphonate class possibly including the tris-(2-chloroethyl)-methyl phosphonates.

The use of sodium hydride as the base for proton abstraction gave an insoluble white solid in a highly exothermic reaction which appeared to be the salt of the carboxylic acid CXXII. It is possible that this salt could have arisen via the ketene, CXXI. See fig. 62.

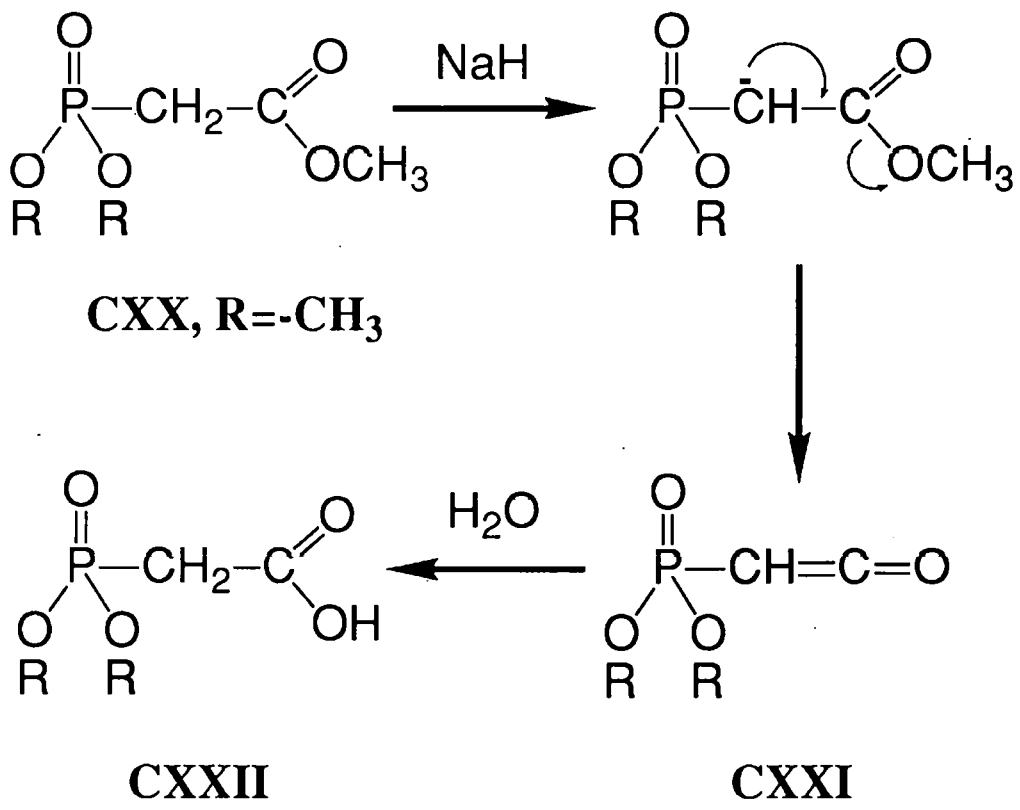


Fig. 62

This was supported by evidence from the ^1H NMR spectrum of CXXII, R = $-CH_3$ which showed that the methylene doublet (split by phosphorus) had moved downfield by 12Hz (d, $^2J_{H,P}$ = 22Hz). The product isolated from the action of sodium hydride on dimethyl ethylphosphonoacetate gave absorption peaks at 1580cm^{-1} and at 1460cm^{-1} in the infrared which can be assigned to the formation of the phosphonoacetate salt of the acid CXXII.

The existence of CXXII was further confirmed by the formation of a 2-oxazoline derivative, CXXIV, upon reaction with 2-amino-2-methylpropan-1-ol. However, further addition of base to the protected carboxylate, which would be expected to yield its anion did not react with 2-chloroethyl tosylate. See fig. 63.

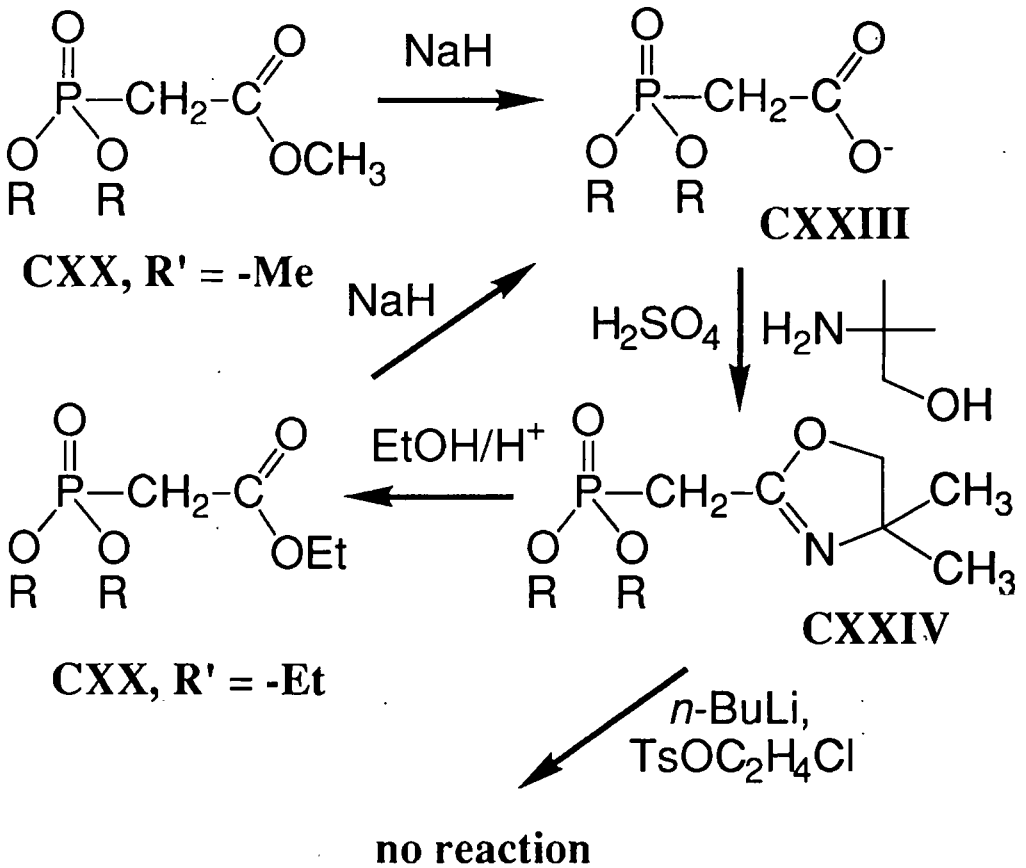


Fig. 63

Another agent used for the attempted direct 2-chloroethyl addition was 1-bromo-2-chloroethane. It was hoped that the differential activity of the two halides would be sufficient to specifically yield the 2-chloroethyl adduct as the major product. However, it proved impossible to isolate a product that had spectral characteristics compatible with the desired product (See section II.2.3).

It was likely that the whole scheme had an implicit difficulty which would become apparent upon addition of the second equivalent of base to the mono-2-chloroethyl adduct, CXVIII.

Upon exposure to *n*-butyllithium, intramolecular substitution of chloride would be expected to form a cyclopropane derivative. For this reason, the first addition requires a 'latent' or protected chlorine function such as with an alcohol, which can be later converted into a chlorine after the addition of a second chloroethyl group. Following the failure to synthesise CXVIII and as the scheme outlined above is cumbersome, this approach was not continued.

II.3.2 Indirect Addition of a 2-chloroethyl Group

Addition of latent 2-chloroethyl groups provide a method for introducing both groups to give the basic structure which can then be chlorinated to yield the mustard. The easiest group to use as a latent chlorine is an alcohol moiety. Any of the usual chlorinating agents, such as hydrochloric acid and zinc chloride, can be expected to chlorinate the alcohols once attached to the phosphorus, thus introducing a 2-hydroxyethyl group to the phosphonate skeleton can be expected to be the problem. See Fig. 64.

The first method of alcohol introduction tried was using ethylene oxide. After many attempts, it was found best to prepare the oxide by the action of 2-chloroethanol onto sodium hydroxide and force it directly into a second vessel containing the pre-formed phosphonate anion. (A small reservoir of liquid oxide was found necessary to avoid any 'suck-back' caused during different stages of the oxide preparation).

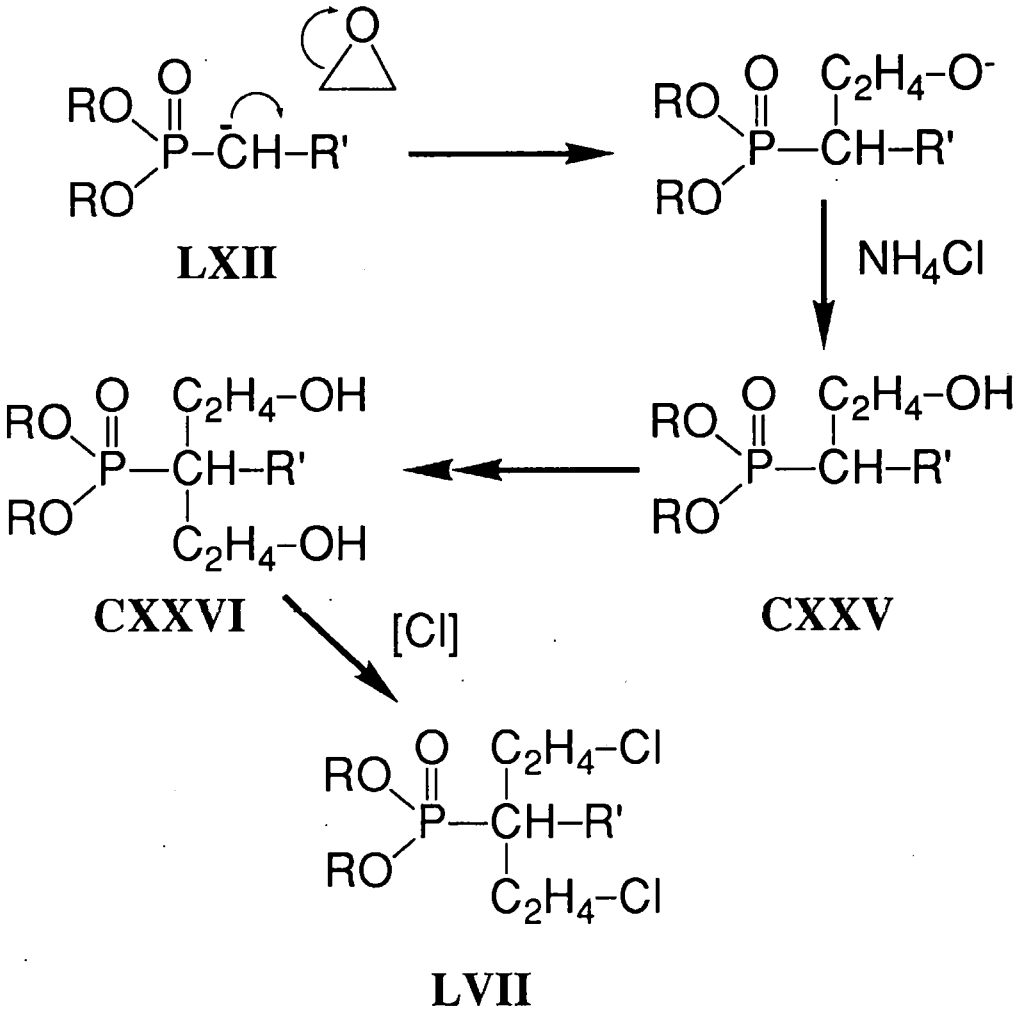


Fig. 64

However, this strategy also suffered from the apparent unreactivity of the phosphonate anion; no evidence for the addition product was seen despite many varied attempts.

Alternatively, addition of a protected 2-bromoethanol was also tried as a method for the introduction of a latent 2-chloroethyl group. The alcohol function was protected by the addition of dihydropyran to give the base stable 2-(2-bromoethoxy)tetrahydropyran. (See CII in fig. 49).

No reaction was observed with this reagent. This may have been due to some form of intra-molecular interaction of the protected bromoethanol (similar to that proposed for the organometallic compounds discussed in section II.2.1.4).

II.4 FURTHER STUDIES

It would not be prudent to postulate that it is impossible to synthesise these compounds from the results of this project. It does, perhaps, point towards the necessity for a different approach to avoid the pit-falls of elimination, steric hindrance and difficulties in purification that were encountered with this work.

One further method, which was not tried, is to make the protected 1,5-dihydroxypentan-3-one and the phosphonate anion to attack it before de-protecting the alcohols. This scheme is outlined in fig. 65.

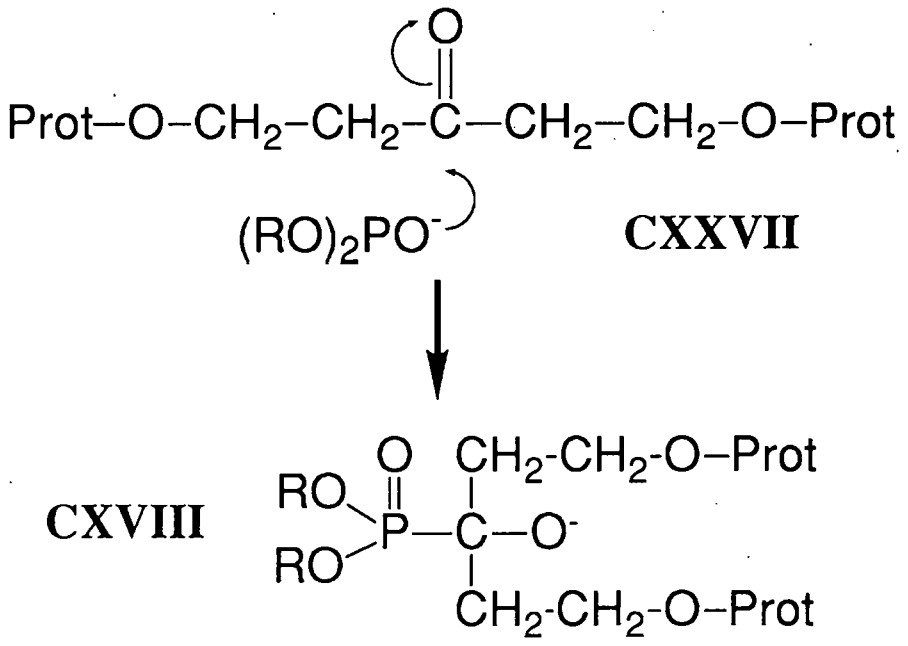


Fig. 65

Even as advances in methods of administering the drugs changes, such as monoclonal antibody tagging for target tissue specificity, the need for anti-

tumour drugs is not threatened and so the development of these compounds would still be a useful exercise.

However, further development of the schemes tried in this work does not suggest any obvious course; the difficulties encountered are somewhat intrinsic to the chemistry involved. The most immediate problem is the necessary improvement in purification techniques so as to be able to isolate the desired product from the inevitable mass of phosphoryl by-products. But crucially, if the full potential of these compounds is to be determined, a useful general synthetic route is needed to obtain the many analogues required for the design of a kinetically controllable drug.

Part III: Experimental

Experimental

III.1 Introduction

This section includes the experimental details and physical evidence of the synthesis and purification of compounds prepared and described in section II. The procedure outlined is of the optimal method obtained for each group of compounds. The yield of each compound is of the final product on which the spectroscopic data was obtained.

Carbon and hydrogen microanalyses were carried out on a Perkin Elmer 240C Elemental Analyser. Infrared spectra were recorded using a Unicam SP1050 spectrophotometer and were obtained using either pure liquids or, for solid products, suspended in a nujol mull sandwiched between sodium chloride plates. Proton nuclear magnetic resonance spectra at 60 MHz were obtained using a Pye Unicam R12B continuous wave instrument while proton spectra at 89.56 MHz, carbon-13 spectra at 22.50 MHz and phosphorus-32 at 36.26 MHz were all carried out at room temperature on a Jeol FX90Q Fourier transform machine. All samples were dissolved in deuteriochloroform or deuterium oxide unless otherwise stated. Tetramethylsilane was used as a reference for hydrogen and carbon-13 spectra with a resonance of $\delta 0.00$; phosphoric acid was used as an external reference for phosphorus spectra with a reference resonance of $\delta 0.00$. (Carbon-13 resonance values are given for proton decoupled spectra (COM) but off-resonance decoupling with nuclear Overhauser effect (nOe) was used to determine the number of attached protons). Mass spectra were obtained from the PCMU service at Swansea.

III.2 The Syntheses

The experimental details are given in the order in which they were discussed in section II. For more detail see contents list on page vii. For each class of compound, the method used for the one considered most typical for its class is given in full: other compounds within the same class are then presented with the major differences and their full identification data.

III.2.1 Synthesis of Trihalopentanes

III.2.1.1 1,5-Dichloropentan-3-one (LXXIV)

To a stirred suspension of anhydrous aluminium chloride (300g, 2.5 mol) in dichloromethane (150 cm^3) contained in a cooled (ice/salt bath) three neck flask (3 l), 3-chloropropionyl chloride (127g, 1 mol) in dichloromethane (100 cm^3) was added drop wise over a period of $^1/_2$ hr. The suspension was stirred for a further $^1/_2$ hr. to allow completion of complexation and temperature equilibration. Ethylene, dried by passing over sodium hydroxide and calcium chloride, was bubbled slowly through the suspension for 2-4 hrs. (Infrared spectroscopy was used to follow the reaction). The reaction products were then added slowly to a cooled (ice/salt) mixture of dichloromethane (300 cm^3), hydrochloric acid (1M, 200 cm^3) and ice (200g + extra as needed). (Care was taken with the larger volumes not to let the dichloromethane layer boil). The organic layer was separated, washed with water (3 x 2 l), and dried with anhydrous magnesium sulphate. The solvent was evaporated under reduced pressure to leave the crude 1,5-dichloropentan-3-one as a dark brown oil purification of which proved to be difficult.

Yield: 120.9g, 78%. Elemental analysis found: C, 44.6%; H, 5.97%. $C_5H_8Cl_2O$ requires C, 38.7%; H, 5.1%. IR υ max (cm^{-1}) 1800 (C=O), 670 (C-

Cl anti). ^{1}H NMR (90MHz), ($CDCl_3$) δ3.75 (2H ,t, $^3J_{H,H}$ = 7 Hz, -CH_2-Cl), δ2.95(2H, t, $^3J_{H,H}$ = 7 Hz, -CH_2-CO). ^{13}C NMR (22.5 MHz), ($CDCl_3$) (COM), δ45.3 (-CH_2-Cl), δ 37.9 (-CH_2-CO).

III.2.1.2 Synthesis of bis-(2-Chloroethyl) Alcohols (LXXXIX)

III.2.1.2.1 1,5-Dichloro-3-methylpentan-3-ol

To a suspension of magnesium (28g, 1.2 mol) in freshly dried and distilled diethyl ether (200 cm^3) contained in a cooled (ice/salt bath) three necked flask (3 l), 5% iodomethane in diethyl ether (93 cm^3, 1.5 mol) was added and left until reaction had occurred. After cooling, 1,5-dichloropentan-3-one (800 cm^3 of a 1.876 M solution in dry tetrahydrofuran, 1.5 mol) was added to the Grignard reagent over $^1/_2$ hr. After complete addition the mixture was refluxed for 2 hrs. before quenching with water. The sponge like material was thoroughly washed with diethyl ether and then washed with water (3x 250 cm^3) and dried over anhydrous magnesium sulphate. The product was distilled under reduced pressure (0.02mbar at 60-70°C) to give an initially clear liquid.

Yield: 193.5g, 75.4%. IR υ max (cm^{-1}) 3480 (OH), 720 (C-Cl). ^{1}H NMR (90MHz), ($CDCl_3$), δ3.65 (4H, t, $^3J_{H,H}$ = 6 Hz, Cl-CH_2-), δ2.00 (4H, t, , $^3J_{H,H}$ = 6 Hz, -CH_2-COH), δ1.25 (3H, s, -CH_3). ^{13}C NMR (22.5 MHz), ($CDCl_3$) (COM), δ44.6 (-CH_2-COH), δ40.0 (-CH_2-Cl), δ26.6 (-CH_3).

III.2.1.2.2 Other bis-(2-chloroethyl) alcohols

Similar procedures were carried out to the method described above for 1,5-dichloro-3-methylpentan-3-ol.

1,5-dichloro-3-ethylpentan-3-ol

Yield: 1.25g, 67%. IR υ max (cm^{-1}) 3500 (OH), 720 (C-Cl). ^1H NMR (60MHz), ($CDCl_3$), δ3.60 (4H, t, $^3J_{H,H}$ = 7.5 Hz, $-CH_2-Cl$), δ1.95 (4H, t, $^3J_{H,H}$ = 7.5 Hz, $-CH_2-COH$), δ1.75 (1H, s, -OH), δ1.50 (2H, q, $^3J_{H,H}$ = 7.5 Hz, $-CH_2-CH_3$), δ0.90 (3H, t, $^3J_{H,H}$ = 7.5 Hz, $-CH_3$). ^{13}C NMR (22.5 MHz), ($CDCl_3$) (COM), δ41.4 ($-CH_2-COH$), δ39.8 ($-CH_2-Cl$), δ31.6 ($-CH_2-CH_3$), δ8.0 ($-CH_3$).

1,5-dichloro-3-phenylpentan-3-ol

Yield: 2.3g, 50%. IR υ max (cm^{-1}) 3600 (OH). ^1H NMR (90MHz), ($CDCl_3$), δ7.35 (5H, s, Ph-H), δ3.40 (4H, m, $-CH_2-Cl$), δ2.34 (4H, t, $-CH_2-COH$), δ2.16 (1H, s, disappears with D_2O skake, -OH). ^1H NMR (90MHz), (C_6D_6), δ7.400-7.000 (5H, m, Ph-H), δ3.000 (4H, m, $-CH_2-Cl$), δ1.863 (t, $^3J_{H,H}$ = 7.57 Hz) and δ1.844 (t, $^3J_{H,H}$ = 8.06 Hz) - summing to 4H, $-CH_2-COH$), δ8.04 (1H, s, -OH). ^{13}C NMR (22.5 MHz), ($CDCl_3$) (COM), δ128.7, δ127.1 & δ124.8 (Ph), δ45.7 ($-CH_2-COH$), δ39.9 ($-CH_2-Cl$). m/e M^+, absent, 171, 169 (33%,100%: $PhCOH^+-C_2H_4-Cl$), 105 (60%: $Ph-CO^+$), 91 (26%: $Cl-C_2H_4-CO^+$), 77 (34%: Ph^+), 65, 63 (5%, 17%: $Cl-C_2H_4-CO^+$).

3-butyl-1,5-dichloropentan-3-ol

Yield: 10%. ^1H NMR (60MHz), ($CDCl_3$), δ3.60 (4H, t, $^3J_{H,H}$ = 8 Hz, $-CH_2-Cl$), δ2.00 (4H, t, $^3J_{H,H}$ = 8 Hz, $-CH_2-COH$), δ1.70 (1H, s, -OH), δ1.40 (b) & δ0.90 (b) summing (9H, $-CH_2-CH_2-CH_2-CH_3$).

III.2.1.3 Halogenation of bis-(2-Chloroethyl) Alcohols

III.2.1.3.1 Chlorination: 3-methyl-1,3,5-trichloropentane (XC,R=-CH_3)

Zinc chloride (6.5g, 50 mmol) was slowly added to a stirred volume of concentrated hydrochloric acid (10 cm^3 of a 10M sol., 0.1 mol) and when this had dissolved, 1,5-dichloro-3-methylpentan-3-ol (1.71g, 10 mmol) was added and stirred vigorously for $^1/_2$ hr. To the mixture, dichloromethane (100 cm^3) and water (100 cm^3) were added and the organic layer extracted. This was washed with sodium hydroxide (2 x 50 cm^3 of 1 M sol.) and water (3 x 50 cm^3) before being dried over anhydrous magnesium sulphate and the solvent removed under reduced pressure. Distillation under reduced pressure (60-62°C @ 0.02 mbar) gave a colourless, oily liquid.

Yield: 1.06g, 58%. ^{1}H NMR (60MHz), ($CDCl_3$), δ3.65 (4H, t, $^3J_{H,H}$ = 7 Hz, -CH_2-Cl), δ2.25 (4H, t, $^3J_{H,H}$ = 7 Hz, -CH_2-CH_2Cl), δ1.60 (3H, s, -CH_3).^{13}C NMR (22.5 MHz), ($CDCl_3$) (COM), δ46.6 (-CH_2-CCH_3), δ39.5 (-CH_2-Cl), δ29.4 (-CH_3), not identified (C).

III.2.1.3.2 Other chlorocarbon mustards

3-Ethyl-1,3,5-trichloropentane (XC,R=-C_2H_5)

^{1}H NMR (60MHz), ($CDCl_3$), δ3.70 (4H, t, $^3J_{H,H}$ = 7 Hz, -CH_2-Cl), δ3.25 (2H, q, $^3J_{H,H}$ = 6Hz, -CH_2-CH_3), δ2.00 (4H, t, $^3J_{H,H}$ = 7 Hz, -CH_2-CH_2-Cl), δ1.30 (3H, t, $^3J_{H,H}$ = 6Hz, -CH_3).

3-Phenyl-1,3,5-trichloropentane (XC,R=-Ph)

The solution was stirred vigorously at 90°C for 2 hrs. ^{1}II NMR (60MHz), ($CDCl_3$), δ7.60-7.25 (m, Ph-H), δ3.70 (t, -CH_2-Cl), δ2.75 (t, -CH_2-CPh) (Compound not pure).

III.2.1.3.3 Bromination: 3-bromo-1,5-dichloro-3-methylpentane (CXI,R=-CH_3)

To hydrogen bromide in acetic acid (48%, 20 cm^3) 1,5-dichloro-3-methylpentan-3-ol (24.3g, 0.142 mol) was added and stirred vigorously for 17 hrs. The mixture was then diluted with (100 cm^3) water and the product extracted with dichloromethane (3 x 50 cm^3), washed with sodium hydroxide (2 x 50 cm^3) and water (3 x 50 cm^3) before being dried over anhydrous magnesium sulphate. The product was purified using flash chromotography (1g of product down a column of medium grade silica (40g) and eluted with dichloromethane). Product was collected at R_f 0.4 - 0.5.

Yield: 49%. ^1H NMR (60MHz), ($CDCl_3$), δ3.70 (4H, t, $^3J_{H,H}$ = 8 Hz, -CH_2-Cl), δ2.30 (4H, t, $^3J_{H,H}$ = 8 Hz, -CH_2-CBr), δ1.75 (3H, s, -CH_3). ^{13}C NMR (22.5 MHz), ($CDCl_3$) (COM), δ66.1 (C-Br), δ48.0 (-CH_2-CBr), δ40.7 (-CH_2-Cl), δ31.3 (-CH_3).

III.2.1.4 Alcohol Protected Compounds

III.2.1.4.1 Synthesis of 2-(2-bromoethoxy) tetrahydropyran (CII)

To a magnetically stirred mixture of 2-bromoethanol (63g, 0.5 mol) and dihydropyran (42.0g, 0.5 mol) one drop of concentrated sulphuric acid was added. After 1 hr, water (50 cm^3) was added and the mixture was cooled before extraction with dichloromethane (3 x 50 cm^3). The organic layer was dried over anhydrous magnesium sulphate and the solvent evaporated under reduced pressure.

Yield: 72.25g, 68.9%. IR υ max (cm^{-1}) 1130 & 1080 (ether: asymmetric stretch). ^1H NMR (60MHz), ($CDCl_3$), δ4.95 (1/8H, s, O-CH-O: eq.), δ4.70

(7/8H, s, O-CH-O, ax.), δ4.15-3.40 (6H, m, $-CH_2-O-$:cyclic & $-CH_2-CH_2Br$), δ1.70 (6H, b, $-CH_2-$:cyclic).

III.2.1.4.2 Synthesis of 2-(2-chloroethoxy)tetrahydropyran

To a magnetically stirred mixture of 2-chloroethanol (24g, 0.3 mol) and dihydropyran (25g, 0.3 mol) one drop of concentrated sulphuric acid was added. After about 1 hr, following the exothermic reaction, water (50 cm^3) was added and the mixture cooled before extraction with dichloromethane (3 x 50 cm^3). The organic layer was dried with anhydrous $MgSO_4$ and the solvent removed under reduced pressure.

Yield: 35.04g, 70.8%. ^1H NMR (60MHz), ($CDCl_3$), δ5.3(1/7H,s,0-CH-O:eq.), δ4.70(6/7H, s, O-CH-O,ax.), δ4.15-3.30(6H, m, $-CH_2-O-$:cyclic & $-CH_2-CH_2Cl$), δ1.65 (6H, b, $-CH_2-$:cyclic).

III.2.1.4.3 Addition of *n*-Butyllithium to 2-(2-Bromoethoxy)tetrahydropyran

To a solution of, 2-(2-bromoethoxy)tetrahydropyran (2.1g, 10mmol), *n*-butyllithium (6 cm^3 of a 1.5M solution) was added from a syringe under an atmosphere of dry nitrogen. The resulting pale straw coloured solution was left to stir for 1 hr. Water was added and the mixture extracted using methylene chloride (3x50cm^3), dried over anhydrous magnesium sulphate and filtered before the solvent was removed under reduced pressure.

Yield: 3g. IR υ max (cm^{-1})no alcohol peak seen. ^1H NMR (60MHz), ($CDCl_3$) δ5.00 (b), δ4.70 (b) & δ4.30-δ3.40 (m) (-O-CH), δ2.30 - δ0.90 (m, aliphatic hyrdogens).

III.2.1.4.4 Attempted synthesis of 1,5-dichloro-3-methylpentan-3-ol tosylate (CVIII,R'=-CH_3)

a) To a solution of 4-toluenesulphonyl chloride (1.9g, 10 mmol) in methylene chloride (10 cm^3), 1,5-dichloro-3-methylpentan-3-ol (1.71g, 10 mmol) was added as a solution in methylene chloride (10 cm^3). To this mixture, pyridine (1 cm^3) was added and the mixture refluxed for 2-48 hrs. Samples of the reaction mixture failed to show more than the reactants by ^1H NMR.

b) To a mixture of 4-toluene sulphonyl chloride (0.8g, 5 mmol) and 1,5-dichloro-3-methylpentan-3-ol (1.71g, 10 mmol), sodium hydroxide solution (1.6 cm^3 of a 20% sol. in H_2O) was added while the mixture was stirred. After 10mins another equal volume of 4-toluene sulphonyl chloride (0.8g, 5 mmol) was added and this was followed by addition of further sodium hydroxide solution (1.6 cm^3 of a 20% sol. in H_2O). This was left stirring at room temperature for 48hrs. After this time the mixture was diluted with hexane and washed with sodium hydroxide (3 x 25 cm^3 of a 10% sol). Thin layer chromotography revealed only the starting products at R_f 0.8 and ^1H NMR failed to identify any product.

c) To a solution of 3-bromo-1,5-dichloro-3-methylpentane (2.34g, 10 mmol) in dry dioxane (40 cm^3), a solution of silver *p*-toluenesulphonate (2.79g, 10 mmol) in dry dioxane (50 cm^3) was added as a suspension under an atmosphere of nitrogen and left to stir for 1 hour. After this time a white precipitate had formed suspended in a yellow solution. To the reaction mixture, trimethyl phosphite (0.62g, 5 mmol) was added and the mixture was refluxed for $^1/_2$-48hr. Thin layer chromotography revealed that no reaction of the trimethyl phosphite occured and ^1H NMR failed to identify any product.

III.2.2 Attempted Synthesis of Carbon Mustard Phosphonates

III.2.2.1 Arbusov Reaction: with Dialkyl phosphite and Trihalide

A mixture of 1,3,5-trichloro-3-methylpentane (1.85g, 10 mmol) and trimethyl phosphite (13.20g, 0.1 mol) was refluxed for $^1/_2$ - 48hrs. Product isolated was dimethyl methanephosphonate, the product of the thermal rearrangement of trimethyl phosphite.

Yield: 3.0g, 22%. ^{1}H NMR (60MHz), ($CDCl_3$), δ3.75(6H, d, $J_{P,H}$ = 12Hz, -O-CH_3), δ1.45(3H, d, $J_{P,H}$ = 18Hz).

Similar results were obtained using 3-bromo-1,5-dichloro-3-methylpentane after refluxing for up to 16hr. This reaction produced more side-products than when using 1,3,5-trichloro-3-methylpentane.

III.2.2.2 Reaction of Phosphite Anion and Trihalide

To a stirred solution of dimethyl phosphite (1.1g, 10 mmol) in dried tetrahydrofuran (5 cm^3), *n*-butyllithium (4.5 cm^3 in hexane, 2.5M sol., 10 mmol) was added under dried nitrogen over a period of $^1/_2$ hr. A white precipitate formed. To this, silver tosylate (2.79g, 10 mmol) was added and the precipitate turned black over 5 mins. 1,3,5-trichloro-3-methylpentane (1.85g, 10 mmol) was added and refluxed for 1-24hr but no visible change occurred and no new products were identified using thin layer chromotography.

III.2.2.3 Attempted Formation of Trihalide Grignard

To a magnetically stirred suspension of magnesium (~1g, 50mmol) in sodium dried diethyl ether (20 cm^3), 3-bromo-1,5-dichloro-3-methyl penatane (2.34g,

10mmol) was added under an atmosphere of dry nitrogen. The mixture was left to stir for up to 12 hrs with no evidence for Grignard formation being seen. In some of the variations to this reaction described below, evidence of decomposition products was occasionally found in the ^1H NMR as well as unreacted trihalide.

Variations to the above method in an attempt to form the Grignard reagent of a trihalide include: the use of 3-methyl-1,3,5-trichloropentane instead of 3-bromo-1,5-dichloro-3-methyl pentane; the addition of iodine; the addition of 1,2-dibromoethane; the use of dry tetrahydropyran; and initially pre-drying the trihalide before addition.

III.2.2.4 Dimethyl Phosphonate and 1,5-Dichloro-3-Methyl-3-Tosylate

To a solution of 3-bromo-1,5-dichloro-3-methylpentane(2.34g, 10mmol) in dry dioxane (40 cm^3), silver *p*-toluenesulphonate (2.79g, 10mmol) in dioxane (50 cm^3) was added as a suspension and the solution left to stir for 12hrs. The white precipitate formed was filtered off and washed with dichloromethane. The salt was only weakly soluble in water and not at all in organic solvents.

To a solution of dimethyl phosphite (1.1g, 10mmol) in dry tetrahydrofuran (50 cm^3) under an atmosphere of nitrogen, *n*-butyllithium (7 cm^3 of a 1.5M solution in hexane) was added and the mixture left to stir for 12hr. To the straw coloured solution, the white precipitate was added and the mixture left to stir for 24hrs. The reaction was followed using thin layer chromotography and ^1H NMR but no evidence for any reaction having taken place was seen. The white precipitate was never identified.

III.2.3 Reactions over Potassium or Caesium Fluoride

III.2.3.1 Alkyl Phosphonates

2-Propan-2-ol Dimethyl Phosphonate

To a suspension of potassium fluoride (2.9g, 50mmol) in anhydrous acetone ($10cm^3$, excess), dimethyl phosphite (1.1g, 10mmol) was added and the mixture stirred overnight. The solution was then diluted using methylene chloride, the potassium fluoride filtered off and then dried over anhydrous magnesium sulphate before the solvent was partially removed under reduced pressure. The remaining liquid was allowed to crystallise on standing.

Yield: 1.67g, 97%. Melting point 65-68°C. IR υ max (cm^{-1}) 3320 (broad, OH). ^1H NMR (90MHz), ($CDCl_3$), δ4.40 (H, b, -OH, disappeared with D_2O shake), δ3.86 (6H, d, *J*=10Hz, $-OCH_3$), δ1.44 (6H, d, *J*=15.6Hz, $-CH_3$). ^{13}C NMR (22.5 MHz), ($CDCl_3$) (COM), δ74.5 (d, *J*=163Hz, *q*-C), δ63.7 (d, *J*=7.7Hz, $-OCH_3$), δ25.0 (d, *J*=5.1Hz, $-CH_3$) - both the latter two peaks resolve to quartets in the NOE spectrum.. ^{31}P NMR ($CDCl_3$) δ25.57 (d, *J*=19.54Hz, P-H).

Dimethyl Phosphite and Butanone

To a mixture of dimethyl phosphite (5.5g, 50mmol) and potassium fluoride (5.8g, 0.1mol), butanone (3.6g, 50mmol) was added and gently refluxed for 18hr. The reaction mixture was extracted using methylene chloride and after drying over anhydrous magnessium sulphate, the solvent was removed under reduced pressure.

Yield: 2.16g, 2.2%. IR υ max (cm^{-1}) 3350 (broad, OH). ^1H NMR (60MHz), ($CDCl_3$) δ3.80 (d, *J*=12Hz, $-OCH_3$), δ3.40 (s, $-CH_3$), δ2.95 (b, -OH, disappears on D_2O shake), δ1.80 (m, $-CH_2$), δ1.35 (d, *J*=16Hz, $-CH_3$), δ0.95 (t, *J*=7Hz, $-CH_2-CH_3$).

Dimethyl Phosphite and 3-Pentanone

To a mixture of dimethyl phosphite (5.5g, 50mmol) and potassium fluoride (5.8g, 0.1mol), 3-pentanone (4.3g, 50mmol) was added and gently refluxed for 48hr. The reaction mixture was extracted using methylene chloride and after drying over anhydrous magnesium sulphate, the solvent was removed under reduced pressure.

Yield: 2.89g, 32%. IR υ max (cm^{-1}) 3320 (broad, OH). ^1H NMR (60MHz), ($CDCl_3$) δ3.75 (d, J=12Hz, $-OCH_3$), δ1.80 (m, $-CH_2-$), δ1.05 (t, J=7Hz, $-CH_3$).

III.2.3.2 With 2-Chloroethyl Containing Compounds

Phosphite and 1,5-Dichloropentan-3-one

To a suspension of potassium fluoride (11.6g, 0.2 mol) in dimethyl phosphite (5.5g, 50 mmol), 1,5-dichloropentan-3-one (7.75g, 50mmonl) was added and allowed to reflux for several hours. The mixture was then extracted using methylene chloride and after drying and removal of the solvent was passed down a fine silica chromatography column. Fractions were collected and identified using ^1H NMR.

Fraction at R_f 0.2: ^1H NMR (60MHz), ($CDCl_3$), δ7.30(s), δ3.75(d, J=12Hz), δ0.60(s); R_f 0.7: δ4.55(broad doublet), δ3.75(d,J=12Hz), δ3.20(d, J=12Hz), δ2.8-δ0.7(broad multiplet).

Phosphite and 1,5-Dichloropentan-3-one with *n*-Butyllithium

To a magnetically stirred solution of dimethyl benzyl phosphonate (2g, 10 mmol) in dry tetrahydrofuran (30 cm^3) under a dry nitrogen atmosphere, *n*-butyllithium in hexane (15 cm^3 of a 1.55M sol, 10 mmol) was added. After 30mins, the solution was cooled to -10°C and 1,5-dichloropentan-3-one (1.55g, 10mmol) was added and stirred for several hours. The mixture was

then extracted using methylene chloride and after drying and removal of the solvent was passed down a fine silica chromatography column. Fractions were collected and identified using ^1H NMR.

Fraction at R_f 0.3: ^1H NMR (60MHz), ($CDCl_3$), δ6.4-δ5.5 (bm, alkene), δ3.80(d, *J*=12Hz), δ3.70(d, *J*=12Hz), δ3.2-δ2.8(m), δ1.8-δ1.2(b), δ0.95(d, (unequal).

Dimethyl Phosphite and 3-Bromo-1,5-dichloro-3-methylpentane

To a suspension of potassium fluoride (2.9g, 50 mmol) in dimethyl phosphite (1.1g, 10 mmol), 3-bromo-1,5-dichloro-3-methylpentane (2.54g, 10 mmol) was added and the paste stirred for 5 hr. This was extracted with dichloromethane (3 x 25 cm^3), filtered and the solvent dried with magnesium sulphate and removed under reduced pressure. The mixture isolated gave an identical ^1H NMR spectrum to the starting compounds plus the associated break-down products of the 2-chloroethyl groups.

Trimethyl Phosphite and 3-Chloropropionyl Chloride

To a cooled solution of trimethyl phosphite (4.25g, 50mmol) and potassium fluoride (2.9g, 50 mmol) 3-chloropropionyl chloride (6.35g, 50mmol) was added and left for 1 hour. The products were extracted using methylene chloride (3 x 25 cm^3) and dried over magnesium sulphate before having the solvent removed under reduced pressure.

IR υ max (cm^{-1}) 1800 (acyl chloride, C=O), 1735. ^1H NMR (60MHz), ($CDCl_3$), δ6.6 – δ6.0(m)(alkene protons), δ3.80(m), δ3.40(triplet of doublets), δ2.80(t), δ2.20(s).

Triethyl Phosphite and 3-Chloropropionyl Chloride

A similar method to trimethyl phosphite and 3-chloropropionyl chloride was used using triethyl phosphite insted of trimethyl phosphite.

IR υ max (cm^{-1}) 3400-2800 (O-H stretch), 1825 (C=O, anhydride) 1735 (C=O, alkyl acid stretch). ^1H NMR (60MHz), ($CDCl_3$), δ4.20 (q, *J*=6Hz, -O-CH_2-), δ3.70(t, *J*=6Hz, Cl-CH_2-), δ2.80(t, -CH_2-CO), δ1.30(t, *J*=6Hz, -CH_3). ^{13}C NMR (22.5 MHz), ($CDCl_3$) (COM), δ173 (-CO_2-, not split in the NOE), δ64 & δ62 (-CH_3, split into a quartet in the NOE), δ39.2 (-CH_2-CO, split into a triplet in the NOE), δ37.5 (-CH_2-Cl, split into a triplet in the NOE), δ16.4(-O-CH_2-, split into a quartet in the NOE).

1,5-Dichloropentan-3-one alone

1,5-dichloropentan-3-one (2.54g, 10 mmol) was added to potassium fluoride (2.9g, 50 mmol) and left for 1 hour. The reaction flask became hot. The contents were then extracted with methylene chloride and the solvent removed under reduced pressure after first drying with anhydrous magnesium sulphate. IR υ max (cm^{-1}) 1710-1680 (C=O, unsaturated ketone). ^1H NMR (60MHz), ($CDCl_3$), δ6.20 (m), δ5.90(m) (alkene), plus unresolved peaks between δ4.50 & δ0.60.

III.2.4 Preparation of the Phosphonates

III.2.4.1 Dimethyl Benzyl Phosphonate

To a magnetically stirred volume of benzyl bromide (171.04g, 1 mol) in acetonitrile (200 cm^3), trimethyl phosphite (62.04g, 0.5 mols) was added and refluxed vigorously for 16 hours. The reaction mixture was then distilled under reduced pressure (1 mbar at 110-120°C) to give an oily, colourless liquid. The dimethyl benzyl phosphonate was identified by elemental analysis, ^1H and ^{31}P NMR.

Yield: (after two distillations) 0.67g, 20%. Elemental analysis found: C,53.68%; H, 6.67%. $C_9H_{13}O_3P$ requires C, 54.0%; H, 6.5%. ^1H NMR (60MHz), ($CDCl_3$) δ7.30 (5H, s, -Ph), δ3.65 (6H, d, $^3J_{H,H}$ = 10Hz, -OCH3), δ3.15 (2H, d, J_{PCH} = 21Hz, P-CH). ^{13}C NMR ($CDCl_3$) (COM), δ129.8-126.8 (6C, m, -Ph), δ52.79 (2C, d, J = 7.8Hz, $-OCH_3$), δ32.69 (1C, d, Jcp = 137.2Hz, $P-CH_2$). ^{31}P NMR ($CDCl_3$) δ24.29 (m,P).

III.2.4.2 Other Phosphonates

The method used was similar to that used for diethyl benzyl phosphonate, except that the solvents and times varied.

Diethyl Benzyl Phosphonate

Solvent: Acetonitrile. Reflux time: 64 hrs. Fraction: 0.3 bar @ 22-24°C. Yield: 21.0g, 46%. ^1H NMR (60MHz), ($CDCl_3$) δ7.30 (5H, s, -Ph), δ4.00 (4H, dq, J_{POCH} = 8Hz, $^3J_{H,H}$ = 8Hz, $-OCH_2-$), δ3.20 (2H, d, J_{PCH} = 20Hz, $P-CH_2$), δ1.30(6H, dq, J_{POCCH} = 2Hz, $^3J_{H,H}$ = 8Hz, $-CH_3$). ^{31}P NMR ($CDCl_3$) δ2.15 (s,P).

Dimethyl Ethyl Phosphonate

Solvent: 20 fold excess of iodoethane. Reflux time: 2 hrs. Fraction: 0.3 bar @ 22-24°C. **Yield**: 0.41g, 30%. ^1H NMR (60MHz), ($CDCl_3$) δ3.75 (6H, d, $J_{P,H}$ = 10Hz, $P-O-CH_3$), δ1.50(3H, d, $J_{P,H}$ = 18Hz, $P-CH_3$).

Dimethyl Ethyl Phosphonoacetate

Solvent: dioxane. Reflux time: 1 hr. Fraction: 0.5 bar @ 104-108°C. **Yield**: 1.36g, 85%. ^1H NMR (60MHz), ($CDCl_3$) δ4.20 (4H, dq, $^3J_{H,H}$ = 7Hz, $-CH_2$),

δ3.80 (6H, t, $J_{P,H}$ = 12Hz, P-O-CH_3), δ3.00 (2H, d, $J_{P,H}$ = 22Hz, P-CH_2), δ1.30 (3H, t, $^3J_{H,H}$ = 7Hz, -CH_3).

Triethyl Phosphonate

Solvent: acetonitrile. Reflux time: 31 hrs. Fraction: 0.3 bar @ 22-24°C. **Yield:** (after two distillations) 1.10g, 33%. IR υ max (cm^{-1}) 1280 (C-P stretch), 1250 and 1230 (P=O stretch), 1030 (P-O-C stretch). ^1H NMR (90MHz), δ4.10 (4H, dq, $J_{P,H}$ = 7Hz, $J_{H,H}$ = 7Hz, O-CH_2-CH_3), δ1.7 (2H, dq, $J_{P,H}$ = 18Hz, $^3J_{H,H}$ = 7Hz, P-CH_2-), δ1.30 (6H, t, $^3J_{H,H}$ = 7Hz, -CH_3), δ1.10 (3H, t, $^3J_{H,H}$ = 7Hz, P-CH_2-CH_3).

Triethyl Phosphonoacetate

Solvent: dioxane. Reflux time: 4 hrs. Fraction: 0.5 bar @ 100-110°C. **Yield:** 20.0g, 92%. ^1H NMR (60MHz), ($CDCl_3$) δ4.15 (4H, q, $^3J_{H,H}$ = 7Hz, C-O-CH_2), δ4.10 (4H, q, $^3J_{H,H}$ = 7Hz, P-O-CH_2), δ2.90 (2H, d, $^3J_{H,H}$ = 21Hz, P-CH_2), δ1.35 (6H, t, $^3J_{H,H}$ = 7Hz, -CH_3), δ1.30 (3H, t, $^3J_{H,H}$ = 7Hz, -CH_3).

Trimethyl Phosphonate

Solvent: tetrahydrofuran. Reflux time: $^1/_2$ hr. Fraction: Methylene chloride extraction after filtering. **Yield:** 0.90g, 10%. ^1H NMR (90MHz) ($CDCl_3$), δ3.75 (6H, d, $J_{P,H}$ = 12Hz, P-O-CH_3), δ1.5 (3H, d, $J_{P,H}$ = 18Hz, P-CH_3).

III.2.4.3 Electrophillic Attack on Phosphonate Anions

III.2.4.3.1 Phosphonate anion formation with *n*-BuLi

To a magnetically stirred solution of dimethyl benzyl phosphonate (2g, 10 mmol) in dry tetrahydrofuran (30 cm^3) under a dry nitrogen atmosphere, *n*-butyllithium in hexane (15 cm^3 of a 1.55M sol, 10 mmol) was added. After

30mins, the solution was cooled to -10°C and was then ready for further addition of the electrophile.

Phosphate anions were made using a similar method with *t*-butyllithium.

III.2.4.3.2 Electrophilic attack with ethylene oxide

Ethylene oxide was either injected into the reaction mixture from a commercially acquired cylinder or from its remote synthesis by slowly dropping 2-chloroethanol onto sodium hydroxide and injected under its own pressure: it was first dried before being injected by either route of synthesis. To a magnetically stirred solution of pre-formed dimethyl benzyl phosphonate anion under a dry nitrogen atmosphere, dry ethylene oxide gas was gently bubbled through for $^1/_2$ hr. Ammonium chloride (5g, 0.1mol) was added and then the solution extracted with sodium dried diethyl ether which was later removed under reduced pressure.

Column Chromatography: 30g fine silica, 20 x 10 cm^3 samples: R_f 0.4-0.5 gave ^1H NMR (60MHz), ($CDCl_3$), δ3.45(s) - partially lost with D_2O shake, (dimethyl benzyl phosphonate and *n*-butyl compounds).

III.2.4.3.3 Electrophilic Attack with 1-Bromo-2-chloroethane

To a magnetically stirred solution of dimethyl benzyl phosphonate anion (2g, 10 mmol) in freshly distilled and dried diethyl ether (20 cm^3) over ice and under an atmosphere of dry nitrogen, 1-bromo-2-chloroethane (1.43g, 10 mmol) was added and was left to warm up gradually to room temperature for 24 hr. The reaction product was extracted using dichloromethane (3 x 20 cm^3)

and was washed with dilute ammonium chloride solution (3 x 20 cm^3 of a 1M sol), dried over magnesium sulphate and the solvent removed under reduced pressure. Fine silica column chromotography was then used to separate the three bands seen with thin layer chromotography at R_f 0.4, 0.5 and 0.7.

Band 1: ^1H NMR (60MHz), ($CDCl_3$), δ1.0 - 2.0 (m) and signals due to dimethyl benzyl phosphonate. Bands 2 & 3 were unidentifiable.

III.2.4.3.4 Electrophilic attack with 2-tosyl-1-chloroethane

To a magnetically stirred solution of trimethyl phosphonoacetate anion (1.82g, 10 mmol) in freshly distilled and dried tetrahydrofuran (50 cm^3) under an atmosphere of dry nitrogen, 2-tosyl-1-chloroethane (2.34g, 10 mmol in 20 cm^3 of tetrahydrofuran) was added and the mixture was left to reflux gently for 20 mins by which time solid had appeared. The reaction product was extracted using diethyl ether (3 x 20 cm^3) and was washed with dilute ammonium chloride solution (3 x 20 cm^3 of a 1M sol), dried over magnesium sulphate and the solvent removed under reduced pressure.

Yield: 1.1g. ^1H NMR (60MHz), ($CDCl_3$), δ7.60(4H, m, attributed to the phenyl protons on the tosyl group), δ4.25(2H,t, $^3J_{H,H}$ = 6Hz, $-CH_2-O-$ of tosylate), δ3.70(2H+,m,hidden $-CH_2-Cl$ of tosylate and unidentified peaks contributing the equivalent of 6H), δ2.45(3H,s,$-CH_3$ of tosylate), δ1.70(2H,m,unidentified).

III.2.4.3.5 Electrophilic attack with 2-(2-bromoethoxy)tetrahydropyran

To a magnetically stirred solution of dimethyl benzyl phosphonate (2.00g, 10 mmol) in freshly distilled and dried tetrahydrofuran (50 cm^3) under an

atmosphere of dry nitrogen, tetramethyelthylenediamine (1.5 cm^3, 10 mmol) was added followed 5mins later by n-butyllithium (6 cm^3 in a 1.5M sol in hexane, 0.3 mmols). To this 2-(2-bromoethoxy)tetrahydropyran (2.1g, 10 mmol) was added and left to stir at room temperature for 2 hrs. Water (10 cm^3 was added and the mixture left for a further 12hrs. The reaction product was extracted using dichloromethane (3 x 20 cm^3) and was dried over magnesium sulphate and the solvent removed under reduced pressure.

Analysis of the reaction product with thin layer chromotography and ^1H NMR revealed heavy contamination with *n*-butyl residues and tetrahydropyran. These could not be seperated and there was no evidence of any other significant products.

III.2.4.3.6 Ethyl Acetate and 2-(2-bromoethoxy)tetrahydropyran

To a magnetically stirred solution of 2-(2-bromoethoxy)tetrahydropyran (4.2g, 0.02mmol) in dry, freshly distilled tetrahydrofuran (20ml) *n*-butyllithium (22.2ml of a 0.9M solution in hexane) was added dropped wise and allowed to stir for 1/2 hr. The mixture was noted to have become hot. Ethyl acetate was added (0.8g, <0.01mmol) and the mixtiure again allowed to stir for 1 hr. The reaction product was extracted using dichloromethane (3 x 20 cm^3) and was dried over magnesium sulphate and the solvent removed under reduced pressure. Fine silica column chromotography was then used to separate the three bands seen with thin layer chromotography at R_f 0.4, and 0.7.

R_f 0.2: Identified as ethyl acetate.

R_f0.7: **Yield**: 2.2g. ^1H NMR (60MHz), ($CDCl_3$), δ5.0-δ4.6(bd)δ, δ4.4-δ3.3(m) (integration of last two peeks gives 5H), δ2.3-δ0.7 (bm, 8H).

Part IV: References

References

1 Joslin,C.A.F. (Ed.). (1982). Cancer Topics and Radiotherapy: pg 36. Pitman, London.

2 Priestman,T.J. (1980). Cancer Chemotherapy - an Introduction: pg 66. Farmitalia Carlo Erba Ltd., Herts.

3 The Therapeutic Index (TI) of a drug is a measure of its toxicity relative to its dose. It is experimentally determined quantitatively in animals; TI = LD50/ED50, where LD50 is the *leathal* dose in 50% of animals under test and ED50 is the *effective* dose in 50% of animals. (More usually ED*90* is used). Thus the higher the TI for a drug, the more safe the drug is to use. Anti-cancer drugs have some of the lowest TI's of the drugs licensed for medicinal use in the UK.

4 Selby,P. (1987). Multiple Myeloma & Related Diseases. *Medicine International;* **40**: 1664.

5 Spiro,S.G. (1986). Lung Cancer - Staging & Treatment, *Medicine International*; **36**: 1470.

6 Commonly used drugs for small cell lung cancer are: methotrexate (antimetabolite), cyclophosphamide (alkylating agent), doxorubicin (antimitotic) and vincristine (vinca alkaloid).

7 A Carcinoma is a malignant tumour of epithelial cell origin.

8 Watson,J.D. & Crick,F.H.C. (1953). A Structure for DNA, *Nature*, Lond.; **171**: 736-738.

9 Dustin,A.P. (1925). Experimental pyknosis, or caryoclastic crisis, through injection of aniline derivatives, *Compt.Rend.Soc.Biochem.*; **93**: 465.

10 Loveless,A. & Revell,S. (1949). New Evidence on the Mode of Action of 'Mitotic Poisons', *Nature*; **164**: 938.

11 Ford,C.E. (1948). Chromosome breakage in nitrogen mustard-treated *Vicia faba* root tip cells, *Proc. 8th Int. Cong. Genet.*: pg. 570.

12 Goldacre,R.J., Loveless,A. & Ross,W.C.J. (1949). Mode of production of Chromosome Abnormalities by the Nitrogen Mustards, *Nature*; **163**: 667-9.

13 Hadow,A., Kon,G.A.R. & Ross,W.C.J. (1948). Effects upon Tumours of Various Haloalkylarylamines, *Nature*; **162**: 824.

14 The para-diphenyl radical is termed 'xenyl'.

15 Elmore,D.T., Gulland,J.M., Jordan,D.U. & Taylor,H.F.W. (1948). The Reaction of Nucleic Acids with Mustard Gas, *Biochem. J.*; **42**: 308.

16 Philips,F.S. (1950). Recent Contributions to the Pharmacology of bis(2-haloethyl)amines & Sulphides. *Pharm. revs.*; **2**: 281-323.

17 Kollar,P.C. (Oct.1943). Report to Ministry of Supply by Robson,J.M.

18 Robson,J.M. (Jul.1943). Biochemical Sub-Committee, Chemical Board, Ministry of Supply.

19 Fell,H.B. & Allsopp,C.B. (Jul.1943). Report to Ministry of Supply.

20 Philips,F.S. (1950). The Nitrogen Mustard-like Action of 2,4,6-tris(Ethylenimino)-s-triazine & other bis(ethylen-imines), *J.Pharm. Exp.Ther.*; **100**: 386.

21 Stora,C.,Brigando,J.,Schwartzkopf,O. & Genin,H (1947). The Action of Mustard Gas on Proteins, *Compt. rend.*; **224**: 1367-9.

22 Gjessing,E.C. & Chanutin,A. (1946). The Effect of Nitrogen Mustards on Viscosity of Thymonucleate, *Cancer Res.*; **6**: 593-8.

23 Brookes,P. & Lawley,P.D. (1963). Evidence for the action of alkylating agents on deoxyribonucleic acid (DNA), *Exp. Cell: Res.Suppl.*; **9**: 521.

24 Loveless,A.,Cook,J. & Wheatley,P. (1965). Recovery from the Lethal Effects of Cross-linking Alkylation, *Nature*; **205**: 980.

25 Priestman,T.J. (1980). Cancer Chemotherapy - an Introduction: pg 34. Farmitalia Carlo Erba Ltd.,Herts.

26 Morrison,R.T. & Boyd,R.N. (1977). Organic Chemistry, 3rd Ed.: pg 464-5. Allyn & Bacon Inc., Lond.

27 Colvin,M., Brundrett,R.B., Kan.M.N.N., Jardine,I. & Fenselau,C. (1976). Alkylating Properties of Phosphor-amide Mustard, *Cancer Research*; **36**: 1121.

28 Williamson,C.E. & Witten,B. (1967). Reaction Mechanism of some Aromatic Nitrogen Mustards, *Cancer Res.*; **27**: 33.

29 Benn,M.H., Kaxmaier,P. Watanatada,C. & Owen,L.N. (1970). The Mechanism of the Reaction of Aryl Nitrogen Mustards with Nucleophiles, *J.C.S.Chem.:Comm.*: 1685.

30 Blandamer,M.J., Golinkin,G.S. & Robertson,R.E. (1969). Mechanism and solvent reorganisation accompanying hydrolyses of anchimerically assisted alkyl halides in water, *J.Amer.Chem.Soc.*; **91(10)**: 2678.

31 Bohme,H. & Sell,K. (1948). Hydrolysis of halogenated ethers and thio ethers in water dioxane mixtures, *Chem.Ber.*; **81**: 123.

32 Goering,H.L. & Howe,K.L. (1957). Solvolysis of cis- and trans-2-chlorocycloalkyl aryl sulphides in 80% aq. ethanol, *J.Amer.Chem.Soc.*; **79**: 6542.

33 Tabushi,I., Tamaru,Y., Yoshida,Z. & Sugimoto,T. (1975). Solvolysis of 2-endo- and 2-exo-chloro-7-thia-bicyclo(2.2.1)heptanes, *J.Amer. Chem.Soc.*; **97(10)**: 2886.

34 Engle,T.W., Zon,G. & Egan,W. (1982). ^{31}P NMR Kinetic Studies of the Intra- and Intermolecular Alkylation Chemistry of Phosphoramide

Mustard & Cognate N-Phosphorylated Derivatives of N,N-Bis(2-chloroethyl)amine, *J.Med.Chem.*; **25**: 1347.

35 Williamson,C.E., Kirby,J.G., Miller,J.I., Sass,S., Kramer,S. P., Seligman,A.M. & Witten,B. (1968). Enzyme alterable Alkylating Agents IX. The Enzymatic Transformation of some Nitrogen Mustards in the Presence of Carbon Dioxide: Implications in Respiration, *Cancer Res.*; **26**: 323.

36 Gomori,G. (1948). Histochemical demonstration of sites of phosphor-amidase activity, *Proc.Soc.Exp.Biol.Med.*; **69**: 407.

37 Phosphorylase is an intracellular enzyme which will cleave P- N bonds; its natural substrates and functions are unknown.

38 Friedman,O.M. & Seligman, A.M. (1954). Preparation of N-phosphorylated derivatives of bis-β-chloroethylamine, *J.Amer. Chem.Soc.*; **76**: 655.

39 A point later shown to be untrue.

40 Seligman,A.M., Nachlas,M.M., Manheimer,L.H., Friedman,O.M. & Wolf,G. (1949). Histochemical demonstration of hydrolytic intracellular enzymes in cancer research, *Ann.Surg.*; **130**: 333.

41 Colvin,M., Padgett,C.A. & Fenselau,C. (1973). Biologically Active Metabolite of Cyclophosphamide, *Cancer Research*; **33**: 915.

42 Alarcon,R.A. & Meinkefer,J. (1971). Formation of the cytotoxic aldehyde acrolein during the 5105 in vitro degradation of cyclophosphamide, *Nature New Biol.*; **233**: 250-2.

43 Struck,R.F., Kirk,M.C., Mellet,L.B., El Dareer,S. & Hill, D.L. (1971). Urinary Metabolites of the Anti-cancer Agent Cyclophosphamide, *Mol.Pharmacol.*; **7**: 519-529.

44 Fenselau,C., Kan,M.N.N., Billets,S. & Colvin,M. (1975). Identification of Phosphorodiamidic Acid Mustard as a Human Metabolite of Cyclophosphamide, *Cancer Research*; **35**: 1453.

45 Jardine,I., Fenselau,C., Appler,M., Kan,M.N.N., Brundrett, R.B. & Colvin,M. (1978). Quantitation by Gas Chromatography-Chemical Ionisation Mass Spectrometry of Cyclophosphamide, Phosphoramide Mustard and Nor-Nitrogen Mustard in the Plasma and Urine of Patients Receiving Cyclophosphamide therapy, *Cancer Res.*; **38**: 408.

46 Proceedings of the symposium on the Metabolism and Mechanism of Action of Cyclophosphamide, *Cancer Treatment Reports*; **60**: 299-525; (1976).

47 Mehta,J.R., Przybylski,M. & Ludlum,D.B. (1980). Alkylation of Guanosine & Deoxyguanosine by Phosphoramide Mustard, *Cancer Research;* **40**: 4183.

48 Vu,V.T., Fenselau,C. & Colvin,O.M. (1981). Identification of the 3 Alkylated Nucleotide Adducts from the Reaction of Guanosine 5'-monophosphate with Phosphoramide Mustard, *J.Am.Chem.Soc.*; **103**: 7362.

49 Lindemann,H. & Harbers,E. (1980). (GE) Interaction of the 3 Alkylating Drugs, Cyclophosphamide, Ifosfamide & Trofosfamide with DNA & DNA-constituents *in vitro*, *Arzneim.-Forsch.*; **30-2(12)**: 2075-80.

50 Erickson,L.C., Ramonas,L.M.,Zaharko,D.S. & Kohn,K.W. (1980). Cytotoxicity & DNA Cross-linking Activity of 4-Sulfidocyclophosphamides in Mouse Leukaemia Cells *in vitro*, *Cancer Res.*; **40**: 4216.

51 Hohorst,H.-J., Draeger,U., Peter,G. & Voelcker,G. (1976). The Problem of Oncostatic Specificity of Cyclophosphamide (NSC-26271): Studies on Reactions that Control the Alkylating and Cyclotoxic Activity, *Cancer Treatment Reports*; **60**: 309.

52 The chemical reactivity of a nitrogen mustard compound can be assessed using the NBP test. (See ref. 58).

53 Another method for determining mustard reactivity is by assessing the rate of chloride ion liberation in aqueous solution. The values are given as the reaction rate.

54 Zon,G., Ludeman,S.M. & Egan,W. (1977). High resolution Nuclear Magnetic Resonance investigations of the chemical stability of cyclophosphamide and related phosphoramidic compounds, *J.Amer.Chem.Soc.*; **99**: 5785.

55 Iley,J. & Shenstone,L. (1983). Unpublished observations.

56 Struck,R.F., Kirk,M.C., Witt,M.H. & Laster,W.,jr. (1975). Isolation and Mass Spectral Identification of Blood Metabolites of Cyclophosphamide: Evidence for Phosphoramide Mustard as the Biologically Active Metabolite, *Biomed. Mass Spectrom*; **2(1)**: 46-52.

57 Microsomes do not exist *in vivo* but are formed artificially following centrifugation of lysed liver tissue. They are believed to contain the enzymes of the rough endoplasmic reticulum (organelle responsible for the metabolism of many drugs) including the P450 oxidation system.

58 Friedman,O.M. & Boger,E. (1961). Colourimetric estimation of nitrogen mustards in aqueous solution using 4-(4'-nitrobenzyl)pyridine (NBP) which allows quantitative determination of the actual alkylating potency of a compound, *Ana.Chem.*; **33**: 906.

59 Zwelling,L.A., Filipski,J. & Kohn,K.W. (1979). Effect of Thiourea on Survival and DNA Cross-link Formation in Cells Treated with Platinum (II) Complexes, L-phenyl-alanine Mustard and bis(2-chloroethyl)methylamine, *Cancer Res.*; **39**: 4989.

60 Friedman,O.M. (1967). Studies of some Newer Phosphoramide Mustards, *Cancer Therap. Rep.*; **51(6)**: 347-57.

61 Montgomery,J.A. & Struck,R.F. (1976). Synthesis and Structure-Activity Relationships of Pre-Activated Analogs of Cyclophosphamide (NSC-26271), *Cancer Treatment Reports*; **60**: 381.

62 Iley,J. & Shenstone,L. (1983). Unpublished observations.

63 Emsley,J. & Row,D. (1976). The Chemistry of Phosphorus: ch.4. Harper & Row, London.

64 Davidson,A.H., Fleming,I., Grayson,J.I., Pearce,A., Snowden,R.L. & Warren,S. (1977). Regioselectivity & Control in Alkene Formation from a Carbonium Ion after Diphenylphosphinoyl Migration, *J.C.S.Perkin I*: 550.

65 Texier-Boulett,F. & Foucaud,A. (1982). A convenient synthesis of dialkyl 1-hydroxyalkanephosphonates using potassium or caesium fluoride without solvent, *Synthesis;* **2**: 165.

66 There is analogous kinetics with antibody binding of antigen. Firth,A. (1983). S322; 13-15: Molecular Immunology; 3rd Ed.: pg 31 (Antiserum Avidity). Open University Press, Milton Keynes.

67 Owen,G.R. & Reese,C.B. (1970). A convenient Preparation of Tetrahydro-4H-pyran-4-one, *J.Chem.Soc.(C)*: 2402.

68 Catch,J.R., Elliott,D.F., Hey,D.H. & Jones,E.R.H. (1948). Halogenated ketones: Part III. The preparation of Bromo methyl ketones by the diazomethane reaction, *J.Chem.Soc.*: 278.

69 Dyke,S.F., Floyd,A.J., Sainsbury,M. & Theobald,R.S. (1978). Organic Spectroscopy; 2nd Ed.: pg 170, Table 32.

70 Morrison,R.T. & Boyd,R.N. (1977). Organic Chemistry, 3rd Ed.: pg 443, problem 13.14. Allyn & Bacon Inc., Lond.

71 Trifluoroacetate.

72 Dyke,S.F., Floyd,A.J., Sainsbury,M. & Theobald,R.S. (1978). Organic Spectroscopy; 2nd Ed.: pg 75, Table 16.

73 Davidson,A.H. & Warren,S. (1976). Sterio- and regio-selectivity in diene synthesis using diphenylphosphinoyl as a migrating functional group, *J.C.S:Chem.Comm.*: 181.

74 Fleming,I., Pearce,A. & Snowdon,R.L. (1976). Controlling the outcome of a carbonium ion reaction: the use of a trimethyl silyl group to dictate the formation of a specific olefin, *J.Chem.Soc:Chem.Comm.*: 182.

75 Whilst this is true for the 1,5-dichloro derivative, 1,5-dibromo-3-flouro-3-triflouromethylpentane has been made by fussion of the ethyl componants under high pressure.[76]

76 Piccardi,P.,Modena,M., Santoro,E. & Edison,M.(1972). The Peroxide addition of 1,1-dibromotetrafluoroethane, 2-ethylene and 2-methyl propane. *J .Chem.Soc. Perkin I:* 1146